University of Alberta

EFFECTS OF NUTRIENT ENRICHMENT ON RECRUITMENT OF AGE-0 FATHEAD MINNOWS (*PIMEPHALES PROMELAS*)

by

SUSAN CATHERINE HEDWIG KIESLING

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **"Effects of Nutrient Enrichment on Recruitment of Age-0 Fathead Minnows (*Pimephales promelas*)"** submitted by **Susan Catherine Hedwig Kiesling** in partial fulfillment of the requirements for the degree of **Master of Science in Environmental Biology and Ecology**.

Dr. W.M. Tonn

Dr. N.E. Stacey

Dr. S.E. Macdonald

Date thesis approved by committee: 27 January 1999

Dedicated to my parents for all their loving support and encouragement.

Abstract:

Effects of Nutrient Enrichment on Recruitment of Age-0 Fathead Minnows (*Pimephales promelas*)

Nutrient loads are predicted to increase for northern Alberta lakes due to changes in land-use and climate. Since survival of age-0 fish is frequently size-selective, increased nutrient loading might affect recruitment through its influence on food availability and growth rates. To assess these effects, I manipulated nutrient concentrations in small boreal ponds and examined mechanisms influencing survival of age-0 fathead minnows (*Pimephales promelas*). Egg production, growth, and survival increased during the growing season in response to increased nutrient loading. In a separate study, overwinter mortality of age-0 fish was strongly size-selective; smaller fish experienced greater mortality than larger fish. Since body size of age-0 fatheads increased in response to nutrient addition, recruitment to age 1 should be enhanced by this perturbation. In the examination of mechanisms influencing survival of age-0 fish, the first evidence of daily increment formation on fathead otoliths is presented.

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1. GENERAL INTRODUCTION

1.1 INTRODUCTION

Natural variation in fish population size continues to be a poorly understood phenomenon despite its central importance in fish population biology and fisheries management (Sissenwine 1984; Fritz et al. 1990). Although adult population dynamics can be attributed, in general, to the processes that act on earlier life-history stages (eggs, larvae and juveniles), the specific mechanisms that control survival in these stages, and thus control recruitment (the addition of young fish to the adult population), remain elusive (Miller et al. 1988; Crowder et al. 1992). Elucidation of recruitment mechanisms in natural fish populations is particularly difficult due to the large number of potential abiotic (e.g., temperature, ice cover, water levels) and biotic (e.g., predation, starvation) factors that can interact to influence fish recruitment (Rice et al. 1987). In recent years, evidence for mechanisms that influence natural fish population variation has been further obscured by the increasing stress placed on such populations due to external perturbations, such as global climate and land-use changes.

Food availability, through its influence on both egg production by adults and mortality of young fish, is one factor that can influence recruitment in fish populations and may be indirectly influenced by both climate and land-use change. In adult fish, an increase in ration can increase the number of eggs laid per spawn and, in some fish, can also increase the number of times a fish spawns within a season (Wootton 1977; Reznick and Yang 1993). In young fish, an increase in ration can increase growth rates and body size, which can, in turn, decrease overall mortality from the two key sources of mortality, starvation and predation. In the early life-history stages, larger fish have

better visual acuity (Fernald 1988) and greater swimming speeds (Yates 1983) than smaller individuals, and therefore they are better able to detect and avoid predators and capture prey (Frank and Leggett 1982; Miller et al. 1988). Such effects of ration size can be important since even small differences in the number of eggs produced or in the mortality rate of early life-history stages can have dramatic impacts on the number of fish that survive to recruit to the adult population (Sissenwine 1984; Bailey and Houde 1989). Factors that affect food availability in an aquatic system may therefore be expected to influence recruitment in fish populations.

Although the abundance of primary food sources for young fish, phytoplankton and zooplankton, naturally fluctuate seasonally and annually, external nutrient inputs can have substantial impacts on the planktonic productivity of aquatic ecosystems. Small scale, aquaculture-based experiments (e.g., Milstein et al. 1995) and large scale, ecosystem-based studies (e.g., Colby et al. 1972; Schindler et al. 1973; LeBrasseur et al. 1978; Stockner and Shortreed 1985) have shown that increased nutrient loading into an aquatic system can significantly increase phytoplankton and zooplankton biomass. Several anthropogenic factors can contribute to increased nutrient loading into aquatic ecosystems, including global warming and land-use practices.

A global warming of 2 - 4°C has been predicted for the coming decades, based on observations of increased concentrations of carbon dioxide in the Earth's atmosphere resulting primarily from the burning of fossil fuels (Hansen et al. 1984). Significant increases in mean seasonal temperatures have already been observed in higher latitudes of North America and Eurasia (Hansen et al. 1996). In response to global warming, climate models predict an increase in intense weather events such as thunderstorms

(Hansen et al. 1991), which should increase above ground runoff and nutrient inputs to lakes (Waggoner 1990).

Increased transport of nitrogen and phosphorus into aquatic ecosystems can also occur as a result of land-use practices, particularly following land-clearing activities, such as logging and agriculture. Removal of vegetation from a terrestrial ecosystem should increase soil erosion and runoff into aquatic ecosystems due to the reduction of soil surface roughness, the vegetation canopy and plant transpiration (Schlesinger 1997). A decrease in the roughness of the soil's surface, due to the removal of vegetation, increases the rate of runoff from the terrestrial ecosystem (Abrahams et al. 1994). A reduction in the vegetation canopy can increase the energy of raindrops that reach the soil surface, increasing soil erosion. Further, since water in the soil is taken up by plant roots for transpiration, when vegetation is removed the water content in soil and, as a result, runoff volume should increase (Schlesinger 1997).

The removal of vegetation should also increase nutrient concentrations in the soil and subsequent runoff. This should occur since plants rapidly take up most nutrients, such as nitrogen and phosphorus, present in the surrounding soil; therefore concentrations of these elements should increase in the soil when vegetation is removed. Fertilizer use in agricultural practices can also increase the concentrations of nitrogen and phosphorus in runoff waters (Sharpley and Smith 1993; Carpenter et al. 1998). As a result, aquatic systems containing logged forests and agriculture in their catchments experience an increase in nutrient loads (Carpenter et al. 1998) which can influence food availability for fish (Colby et al. 1972; Schindler et al. 1973; Stockner and Shortreed 1985).

Several studies have investigated the effects of increased nutrient loads on fish populations. In particular, small-scale aquaculture studies have shown that different combinations of nutrients (in the form of organic and inorganic fertilizers) added to small ponds and lakes enhance juvenile fish growth and survival (Fox et al. 1989; Culver et al. 1993; Myers et al. 1996; Tice et al. 1996). In such studies, nutrient regimes are manipulated to maximize food (phytoplankton and invertebrate standing crop) abundance for juvenile fish. These studies contribute primarily to the understanding of the link between nutrient addition and food available for young fish and, in addition, they provide information on the subsequent influence of food availability on juvenile growth and survival.

Several larger ecosystem-scale studies have also been conducted to observe the responses of most life-history stages to different nutrient regimes. Initial responses of fish populations to nutrient addition have included faster growth, improved condition, and greater juvenile (age-0) fish survival, abundance and production (Colby et al. 1972; Nakashima and Leggett 1975; Mills 1985; Mills and Chalanchuk 1987). The responses and subsequent contribution of the adult stage (production of eggs and predation on early-life-history stages) to recruitment are generally not included in these studies because of the difficulty in sampling the egg stage. Specifically, quantification of number of eggs laid and mortality of this stage have generally relied on estimates (LeBrasseur et al. 1978; Mills 1985). For this same reason, estimation of juvenile stage mortality has been difficult, since determining the initial numbers of young fish produced depends on an accurate method of quantifying the number of eggs that hatch.

In these studies, therefore, the specific mechanisms influencing recruitment in fish have most likely been masked by the complexity of the system.

The unique characteristics of the fathead minnow (*Pimephales promelas*) make it an ideal species for the study of the responses of all life-history stages to nutrient enrichment and the influences of these responses on recruitment variability. Fathead minnows are short-lived (≤ 3 years), small-bodied cyprinids (Markus 1934; Scott and Crossman 1973). In northern Alberta, fathead minnows inhabit shallow lakes and ponds that often lack other fish, in particular piscivores, although they frequently co-occur with other small-bodied species (Robinson and Tonn 1989). This species is easily propagated in small experimental ponds and all life-history stages are easily sampled. The combination of the ecologically significant single-species stocking and easy sampling of fathead minnows provides the opportunity--often missing in other studies--to study the effects of nutrient enrichment on the number of young fish that recruit to the adult population realistically yet without the presence of other confounding factors.

Understanding the impacts of nutrient loading is particularly important for populations of fathead minnows in northern Alberta. In this region, increased runoff resulting from global climate change represents a potential source of enhanced nutrient supply to aquatic ecosystems. In addition, there is concern that rapid increases in forestry activities and agriculture will also increase inputs of nutrients. Since fathead minnow populations in this region are at the northern edge of their distribution (Scott and Crossman 1973), the recruitment rate is likely at the low extreme for this species (Shuter and Post 1990). Changes in nutrient loads and their subsequent effects on food

supplies could, therefore, influence recruitment, and are of particular importance for understanding fish population size and its variability.

In the thesis that follows I will present a series of experiments whose aim is the elucidation of mechanisms influencing recruitment of early life-history stages of fish. In a pond study (Chapter Two), I considered explicitly the influence of nutrient enrichment on the production and survival of age-0 fathead minnows. Thus, the study should also provide insights into potential effects on natural populations resulting from changes in climate and land-use, as discussed above. In a separate enclosure experiment (Chapter Three), I determined if otoliths from age-0 fathead minnows could be used to determine the age and growth rates of fish and thus assist in the determination of mechanisms influencing age-0 fish survival in the pond study. I also conducted an enclosure experiment (Chapter Four) to determine how growth and survival of age-0 fish is influenced by food availability and how growth, and hence body size achieved at the end of the growing season, influences overwinter mortality. In the final chapter, I discuss the implications of the results I obtained from these studies for fathead minnow populations in northern Alberta. I will also discuss the broader relevance of these studies for other fish species whose populations may be subject to similar regional and global-scale perturbations.

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2. THE EFFECTS OF NUTRIENT ENRICHMENT ON RECRUITMENT OF AGE-0 FATHEAD MINNOWS (*PIMEPHALES PROMELAS*)

2.1 INTRODUCTION

Fluctuations in fish population size between years and ecosystems remain poorly understood despite their importance to fish ecology and fisheries management (Sissenwine 1984). In attempts to gain a better understanding of these fluctuations, recent research has focused more on the mechanisms acting on the recruitment of young into fish populations (Post and Prankevicius 1987; Crowder et al. 1992; Cargnelli and Gross 1996; Gleason and Bengtson 1996) and factors that influence these mechanisms (Frank and Leggett 1982; Crecco and Savoy 1985; Donald 1997).

Stages thought to play key roles in recruitment, and thus fish population dynamics, occur during early life-history (egg, larval, and post-larval (early juvenile) stages) (Miller et al. 1988; Bailey and Houde 1989). Since it is in these stages that the greatest numbers are produced and the greatest mortality occurs (Sissenwine 1984), even small fluctuations in egg production or mortality rate should strongly influence the numbers of fish that recruit. Changes in factors such as ecosystem productivity, predator densities or abiotic conditions, which can affect these processes, should consequently affect recruitment. The task of determining which specific factors are most important has been difficult, however (Sissenwine 1984; McGovern and Olney 1996).

In freshwater ecosystems, increased nutrient input, which subsequently increases production up the food chain, has been identified as one factor that can influence fish

population size. Numerous studies have reported increases in phytoplankton and zooplankton, the main food sources for young fish, due to changes in nutrient loads (Colby et al. 1972; Schindler et al. 1973; LeBrasseur et al. 1978; Stockner and Shortreed 1985). Therefore, such changes, through their influence on food availability, could influence recruitment and subsequent year-class strength in fish.

Although small-scale, aquaculture experiments have been conducted to determine how to maximize fish yield through nutrient manipulation (Zhu et al. 1990; Milstein et al. 1995; Myers et al. 1996; Tice et al. 1996), there is increasing concern over large-scale cultural eutrophication of natural aquatic ecosystems (Carpenter et al. 1998). As a result, ecosystem-level studies have been conducted to determine or predict impacts of increases in nutrient loads (e.g., from global warming, forestry and agriculture) on fish populations (Colby et al. 1972; Nakashima and Leggett 1975; Mills 1985; Mills and Chalanchuk 1987; Deegan et al. 1997).

Global warming and an associated increase in precipitation are predicted for the coming decades due to increased CO_2 and other greenhouse gases in the Earth's atmosphere (Hansen et al. 1984). Significant increases in mean seasonal temperatures have been observed in higher latitudes of North America and Eurasia (Hansen et al. 1996). Associated with the predicted increase in precipitation, runoff and nutrient loads to aquatic ecosystems should also increase (Waggoner 1990; Carpenter et al. 1992). Therefore, as a result of global climate change, increased nutrient inputs into aquatic ecosystems could occur, particularly at northern latitudes.

Increased transport of nitrogen and phosphorus into aquatic ecosystems can also occur as a result of human land-use practices, particularly following clear-cut logging

and fertilizer use in agriculture (Sharpley and Smith 1993; Carpenter et al. 1998). Removal of vegetation from a terrestrial ecosystem should increase soil erosion and runoff into aquatic ecosystems due to the reduction of soil surface roughness, the vegetation canopy and plant transpiration (Schlesinger 1997). Since most nutrients in terrestrial ecosystems are retained by vegetation, its removal should increase the amount of nutrients present in runoff water. Fertilizer use in agricultural practices can also increase the concentrations of nitrogen and phosphorus in runoff waters (Sharpley and Smith 1993; Carpenter et al. 1998). Aquatic systems containing logged forests and agriculture in their catchments experience an increase in nutrient loads (Carpenter et al. 1998) which can influence food availability for fish (Colby et al. 1972; Schindler et al. 1973; Stockner and Shortreed 1985).

Although large-scale ecosystem-based studies can document impacts of increased nutrient loads on fish populations, the mechanistic insights obtained are limited. Both the complexity and sizes of natural aquatic ecosystems studied make it difficult to accurately quantify the responses of all life-history stages to nutrient addition. As a result, very few studies have investigated the responses of early life-history stages to nutrient loading and determined their effects on recruitment of young, and hence year-class strength.

In most systems, observation and accurate quantification of eggs from first laid to hatch is particularly difficult. As a result, very few studies have determined the influence of environmental factors, such as nutrient loading and its influence on food availability, on the production and survival of eggs through to hatch (Cargnelli and Gross 1996; Bouwes and Luecke 1997). Several factors related to food availability may

influence the number of eggs produced. Since fecundity can be related to female body size (Wootton 1990), more eggs should be produced in systems with greater food availability because growth rates should be faster, resulting in larger females (Emery and Brown 1978; Kvarnemo 1997).

Some of the variation associated with the female size-fecundity relationship can be explained by the influence of female food rations during the spawning period on the number of eggs produced. If food rations are high, females can convert more oocytes into vitellogenic eggs (ready to be spawned) with the available energy. In contrast, when food rations are low, females will resorb vitellogenic eggs, obtaining extra energy but leaving fewer eggs available to be spawned (Tyler and Dunn 1976; Wootton 1977; Reznick and Yang 1993). In multiple-batch spawners, ration size can also influence how many times a fish can spawn in one season, thus influencing the total number of eggs produced (Wootton 1977).

After spawning, the number of eggs that survive to hatch is primarily influenced by predation (Bouwes and Luecke 1997), including cannibalism from conspecific adults and larger juveniles (Wootton 1990; Vandenbos 1996). Cannibalism may decrease in systems with high food levels since more alternative food would be available (Margulies 1990; Gotceitas and Brown 1993). In addition, since a proportion of available energy in species that exhibit parental care is allocated to this behavior (Wootton 1990), greater food availability may improve the condition and size of the adult caregiver, and thus improve its abilities to clean and defend its nest. Therefore, in systems with greater food availability more eggs should be produced and survive to hatch.

Increased nutrient loading, through its influence on food availability, should also control survival of young fish (larval and post-larval stages) by increasing growth rates and body size. Growth is a particularly important factor regulating survival and subsequent recruitment in young fish because their principal mortality mechanisms, predation and starvation, are highly size-dependent (Rice et al. 1987; Miller et al. 1988). Larger fish are stronger swimmers and therefore better foragers that are better able to escape predation (Miller et al. 1988). Thus, faster growth will reduce the time that young fish spend in a smaller, more vulnerable size-class. In addition, faster growth will reduce the time a fish is vulnerable to gape-limited predation.

To assess the effects of nutrient enrichment on recruitment of young fish, I performed a pond experiment with fathead minnows (*Pimephales promelas*). Nutrients were added to one side each of three divided ponds, and I determined the effects of this treatment on the recruitment of young over a full growing season. In particular, I addressed two main objectives: **1.** Determine the effect of nutrient enrichment on the adult stocked fish and on the subsequent production and survival of eggs; **2.** Determine the effects of nutrient enrichment on recruitment of age-0 fish.

2.2 METHODS

2.2.1 Natural History

The fathead minnow (*Pimephales promelas*) is an ideal species to study the responses of all life-history stages to nutrient enrichment and the possible influences of these responses to recruitment variability. Fathead minnows are short-lived (≤ 3 years), small-bodied cyprinids (Scott and Crossman 1973; Markus 1934). They occur throughout central North America, with their northwest distributional limit at the Alberta-Northwest Territories border (Scott and Crossman 1973). In northern Alberta, fathead minnows inhabit shallow lakes and ponds that often lack other fish, in particular piscivores, although they frequently co-occur with other small-bodied species (Robinson and Tonn 1989).

This species is easily propagated in small experimental ponds. Since adhesive eggs are spawned on the underside of objects, e.g., rocks, boards, logs, lily pads (McMillan and Smith 1974), egg collection and quantification is simple. Adult and fry are also easily collected by trapping and electrofishing. In addition, since fathead minnows commonly occur as the only fish species in boreal ponds (Robinson and Tonn 1989), stocking only fatheads into experimental ponds is directly applicable to their ecology. The combination of easy sampling and the ecologically significant single-species stocking of fathead minnows provides the opportunity--often missing in other studies--to observe the responses of all life history stages to nutrient enrichment in the absence of other confounding factors.

Adult fatheads have a varied diet that may include substantial quantities of plant material (algae and macrophytes) (Scott and Crossman 1973). Other common

components of their diet include zooplankton, small insects, and detritus (Held and Peterka 1974; Tallman et al. 1984; Price et al. 1991). Fathead minnows have also been shown to feed on the eggs and young of their own species (Vandenbos 1996). Although not studied specifically for fathead minnows, larval (<10mm) cyprinid diets include predominantly phytoplankton and rotifers (Mills 1991). As the young fish grow they feed selectively on larger food items, including filamentous algae, diatoms, detritus and small invertebrates (Held and Peterka 1974).

Fathead minnows are protracted, multiple-batch spawners. Spawning commences when water temperatures reach between 15.6°C and 17.8°C (in late May - early June in northern Alberta) and continues until August (Scott and Crossman 1973). During the breeding season, males develop secondary sexual characteristics, including darker body coloration, the development of tubercles and a dorsal pad (Markus 1934). These characteristics assist them in establishing and defending a breeding territory (Markus 1934; Scott and Crossman 1973), in spawning (McMillan 1972), and in the cleaning and aeration of eggs within their nest. Females develop an ovipositor ca. 1 mo. prior to spawning (Flickinger 1969). When released from the females, adhesive and buoyant eggs float up and stick to horizontal nesting surfaces prepared and guarded by the males (Scott and Crossman 1973). Egg sizes range from 1.15 mm (Markus 1934) - 1.33 mm (Wynn-Edwards 1932), depending on female size and habitat quality, and the number of eggs per nest range from 36 – 12,000 (Markus 1934). It takes five days for eggs to hatch at 25°C (McMillan and Smith 1974), longer at cooler temperatures. After spawning, adult mortality is high, particularly for males (approximately 80%) (Markus, 1934).

At hatch, larvae have small mouths, pigmented eyes, and yolk sacs and total lengths and standard lengths range, respectively, from 4.8 – 5.2 mm ($\bar{x}$=5 mm) and 4.6 - 5 mm ($\bar{x}$=4.9 mm) (Buynak and Mohr 1979). Post-larval and juvenile fish remain preferentially in shallow, nearshore waters (personal observation).

2.2.2 Experimental Design

2.2.2.1 Study Site

Research was conducted in three experimental ponds (length: 25 m; width: 13 m; maximum depth: 1.5 m), located in northern Alberta at the Meanook Biological Research Station (MBRS) (54°37'N, 113°35'W). Each pond was divided in half to pair one treatment (nutrient addition) and reference (unmanipulated) pond side. Pond division was accomplished by first constructing a supporting framework for a water impermeable curtain. A trench was dug across the width of each pond at the mid-point and five 10.2 cm x 10.2 cm wooden stakes were pounded into the ground along one side of the trench. Crossbeams, which consisted of three rows of 5.1 cm x 2.5 cm x 10.2 cm stakes aligned length-wise, fencing wire, and polyethylene sheets were attached to the 5 wooden stakes to minimize lateral movement. A polyvinyl curtain was then secured to the top and bottom of the supporting framework. Sufficient curtain existed to drape sideways on the pond bottom and this was buried once the trench was filled in. Prior to filling, all ponds were raked to remove any potential spawning substrate. A pump, whose input hose was covered with a mesh screen (mesh size = 2.5 mm) was used to fill the ponds with water from a natural pond located on site. Spawning substrata (eleven floating wooden boards, 0.2 m x 1.5 m, covered by black tarpaulin and anchored with

bricks) was added around the perimeter of each pond side, within one meter from the shore.

2.2.2.2 Treatment (Nutrient Addition)

Beginning on June 13, 1996, one side of each of the three ponds was treated (nutrient addition), with the opposite side left as an unmanipulated reference. Nutrients (liquid inorganic phosphoric acid (85% H_3PO_4) and dry ammonium nitrate (NH_4NO_3 (34%N))) were added four times (after June 13) throughout the season. Quantities of nutrients were calculated to increase the treatment sides' total phosphorus concentration (TP) five times that of the reference sides' TP and to maintain the ratios of total nitrogen (TN) to TP at approximately 20:1. The added quantities were based on TP and TN concentrations in the reference pond sides immediately prior to addition of nutrients to the treatment pond sides (see 2.2.3.1.1 Abiotic environment). Phosphoric acid was added, on average, at concentrations of 80 µg /L-P and ammonium nitrate at 1200 µg /L-N. Application involved first adding both nutrients separately to two buckets containing 15 L of pond water. These mixtures were then sprayed onto the pond surface and mixed vigorously with canoe paddles.

2.2.2.3 Stocked Fish

As a result of the late spring in 1996, the pond experiment did not begin until June 16. May was colder and wetter than average, which delayed construction of pond dividers and capture of fathead minnows from nearby source ponds for stocking.

During June 15-16 1996, each pond side was stocked with fathead minnows at identical densities (1 fish / m^3 (148 fish / pond side)), and distributions by life-history category (0.36 males: 0.36 females: 0.27 juveniles) and size (0.16 of each large males

and females: 0.20 of each small males and females: 0.13 of each large and small juveniles; see below), which were comparable to densities and distributions found in nature (W.M. Tonn, personal communication). Fish had been collected from a nearby pond (53°15'N, 113°18'W) and sorted into six categories: large (74-77 mm TL) and small males (68-71 mm TL), large (68-71 mm TL) and small females (62-65 mm TL), and large (52-59 mm TL) and small juveniles (40-47 mm TL). Males, females and juveniles received batch-marks subcutaneously with acrylic paint for future differentiation of life-history classes. Secondary sexual characteristics (see 2.2.1 Natural History) were used to distinguish between the three life-history categories; juveniles were identified by their lack of secondary sexual characteristics. Low stocking densities were expected to result in high age-0 fish production as a result of low rates of egg cannibalism (Vandenbos 1996). Therefore, if size-selective mortality influences age-0 fathead minnow recruitment, effects would be most pronounced at high age-0 densities, due to high intra-cohort competition (Vandenbos 1996).

2.2.3 Data Collection

2.2.3.1 Environmental Effects

2.2.3.1.1 Abiotic Environment

I collected water samples weekly from all pond sides to determine phosphorus and nitrogen concentrations. In treatment sides, water samples were collected at least 7 d after nutrient addition, except on July 24, when samples were collected within one hour of nutrient addition. Water samples were collected from all pond halves using a 3.1 L Van Dorn bottle from inshore (ca. < 1 m from shore) and offshore (ca. >3 m from shore) habitats and pooled into one composite sample. Samples to be analyzed for TP,

total dissolved phosphorus (TDP), nitrites and nitrates (NO_2^- + NO_3^-), ammonium (NH_4^+) and total Kjeldahl nitrogen (TKN) were filtered and stored (Prepas and Trew 1983). TP and TDP were determined in duplicate within 7 d using Prepas and Rigler's (1982) modification of Menzel and Corwin's (1965) potassium persulfate method. A Technicon autoanalyzer was used to determine nitrates + nitrites and ammonium concentrations, in triplicate, within 24 hours of water sample collection (Stainton et al. 1977). TKN concentrations were also measured in triplicate using Solorzano's (1969) phenolhypochlorite method. Concentrations of TKN + (NO_2^- + NO_3^-) were used to calculate total nitrogen concentrations.

In addition to nutrients, I measured several other water quality parameters. For dissolved oxygen, biweekly water samples were collected mid-depth (0.75 m depth), at inshore and offshore habitats, with 10 ml syringes (Burke 1962) and analyzed using Carpenter's (1965) modification of the Winkler method. pH was measured biweekly from pond water collected near the pond surface and bottom using a Fisher Digital pH meter (Model 109). Temperature was automatically measured hourly and recorded with data loggers submerged midway (0.75 m) down the water column. Data loggers were deployed in pond one and pond two (treatment sides), which represented the range of conditions (exposure to wind and sun) experienced by all pond sides (personal observation).

2.2.3.1.2 Biotic Environment (Food Availability)

Water samples used to quantify phytoplankton biomass (measured as Chl*a*) were collected biweekly with a 3.1 L Van Dorn bottle at mid-depth (0.75 m). Samples from one inshore and offshore site were pooled for each pond side, filtered, and stored

(Prepas and Trew 1983). Chl*a* concentrations were determined in triplicate, within 2 weeks of collection, using an ethanol extraction and spectrophotometric procedure (Bergmann and Peters 1980). Five deployments of a 10L Schindler-Patalas trap (45 µm mesh) each from one inshore and offshore site per pond side were used to collect zooplankton. Trapped zooplankton were preserved in 4% sugar formalin and later sorted into coarse categories (rotifers, cladocerans and copepods). To determine zooplankton dry weights, samples for three dates (June 26, July 22, August 19) were first rinsed with distilled water for 10 minutes to remove sugar and formalin. Next, each sample was poured through three sieves to divide them into three different size fractions (<153 µm, 153-500 µm, > 500 µm). All size fractions were then filtered onto pre-weighed and pre-dried Millipore 0.45 mm filters, oven dried at 60°C for 24 h, cooled in a dessicator and weighed on a Cahn C-31 microbalance. Data for the three size fractions, pooled from inshore and offshore sites on three sampling dates (June 26, July 22, August 19), were obtained for ponds one and two. Some samples from pond two's treatment side and all samples from pond three were excluded due to the relatively high quantities of non-zooplankton organic and inorganic matter present in the samples.

2.2.3.2 Stocked Fish

To monitor growth and survival of stocked fish, six Gee minnow traps were deployed in the evening and collected in the morning, biweekly, in each pond side. Traps were positioned along the shoreline (ca. < 2 m from shore), equally spaced apart in a single row. After the life-history categories of all fish captured were identified, the

fish were counted, measured (TL), weighed and then returned to their respective pond sides.

2.2.3.3 Egg Stage

Egg production and hatching success was determined by censusing eggs within all batches on nest boards on alternate days. Nest board checks commenced immediately after stocked fish were added to the ponds (ca. June 16). Each nest board was divided into three equal sections and the locations of egg batches were mapped on the boards as they appeared. Eggs were enumerated using a wire grid containing 20 1-cm^2 squares. After placing the grid on top of an egg batch, the number of eggs in the core of the batch was determined by multiplying the number of squares covering eggs by the average number of eggs per square; the average number of eggs per square was previously determined from 20 counts of eggs, in different stages of development, within one square. Eggs located peripherally around the core were counted individually and added to the core number to get the total egg number for the batch.

The absence or presence of pigmentation in the eyes of the embryos differentiates egg developmental stages (Nagel 1976). The first stage is identified by the lack of eye pigmentation, in the second stage eyes are pigmented black, and the final (third) stage is characterized by eyes that appear gold due to retinal development. Eggs would typically hatch within 48 h of reaching the third stage.

To determine the total number of eggs laid in a pond side, new stage-one batches were recorded and censused as they appeared in the alternate-day surveys, and their fates were determined from subsequent surveys. Egg batches were identified as hatched if they disappeared on a sampling date after reaching the third (gold-eyed)

stage. Batches disappearing suddenly (and usually entirely) in the first and second stage were considered cannibalized (Vandenbos 1996). Diseased eggs infected by fungus were also recorded; these eggs would remain on the boards for a number of days but gradually disappeared.

2.2.3.4 Age-0 Fish

Age-0 fish were sampled biweekly in all pond sides, from inshore (ca. < 1 m from shore) and offshore (ca. >3 m from shore) habitats, by electrofishing. During each sampling, five 1-m sweeps and five 2-m sweeps were conducted inshore and offshore, respectively, with an electrofishing unit and dipnet. Inshore and offshore samples, collected at similar points along the shoreline, were pooled, resulting in five samples per pond side. Captured fry were counted and returned to the ponds, except for a sample of ca. 30 fry per pond, which were measured to the nearest 1 mm and preserved in 95% ethanol for later otolith analysis (see Chapter Three). At the end of the season (September 1996), ponds were drained to a maximum depth of 0.6 m and all surviving fish were removed using a combination of trapping and seining to provide final measurements of density, growth and survival. Six Gee-minnow traps were set overnight in each pond side and all captured fish retrieved the following morning were preserved in 95% ethanol. After trapping, seining was conducted. A minimum of three consecutive seine hauls containing less than 2 fish was used as a standardized measure to stop seining in each pond side. All fish caught were euthanized (MS-222) and preserved in 95% ethanol. The fish were later counted and subsampled for measurements of total length, standard length, body depth and weight.

2.2.4 Data Analysis

Repeated measures analysis of variance (ANOVA) was used to compare among treatment and reference pond sides data (nutrient concentrations, abiotic and biotic environment, number of eggs laid and hatched between June 19 – July 5 and July 6 - August 5, lengths and numbers of stocked fish, and densities of age-0 fish) sampled throughout the season (June – September, 1996). Treatment (fixed factor), date (repeated measure (fixed) factor), and pond number (random factor) were used as the main effects (Zar 1984). Paired *t*-tests (two-tailed) were used to compare the total number, proportion and condition of age-0 fish that survived to the end of the season between the treatment and reference pond sides. Data for the proportion of age-0 fish that survived were arcsine square root transformed (Zar 1984). The Kolmogorov-Smirnov (K-S) two-sample test was used to compare length-frequency distributions of age-0 fish sampled both during and at the end of the season (September 8) between treatment and reference pond sides (Sokal and Rolf 1981). Data analysis was conducted using the SPSS Base 8.0 statistical package (SPSS 1997). A significance level of $P<0.05$ was used for all tests.

2.3 RESULTS

2.3.1 Environmental Effects

2.3.1.1 Abiotic Environment

As a result of nutrient addition, TDP and nitrates + nitrites concentrations were greater in treatment than in reference sides throughout the season (Table 2-1). Average concentrations of TP were only 2.2 times greater for treatment compared to reference sides (Table 2-1). The July 24 samples, which were collected within 1h of nutrient addition, produced treatment TP concentrations almost 9x greater than reference pond side concentrations (238 µg/L vs. 26.5 µg/L, respectively). Ratios of TN:TP were, on average, 18:1 for treatment sides and 24:1 in reference sides.

Dissolved oxygen was not different between the treatment and reference sides (Table 2-2). pH was alkaline in both treatment and reference sides, ranging from 7.9 to 8.8 (Table 2-2). pH measured near the pond surface and bottom was not different between treatment and reference pond sides throughout the season (Table 2-2).

Mid-depth pond temperatures (Fig. 2-1) ranged from 11.6°C to 22.9°C between June 6 and September 9, 1996. At the beginning of the spawning season temperatures rose from 14.7°C (June 20) to 21.1°C (July 4), subsequently dropping to 17.0°C due to cool and rainy weather, and rising again to 21.1°C on July 15th. Temperature continued to oscillate until the end of August, dropping to 12.0°C by September 10th, immediately prior to draining ponds. Temperatures were not different between pond one and two treatment pond sides (Fig. 2-1).

2.3.1.2. Biotic Environment (Food Availability)

Observed differences in phytoplankton biomass (indicated by Chl*a*) between the treatment and reference pond sides occurred as early as June 26, within two weeks of the first (June 13) nutrient application. Following nutrient addition, phytoplankton biomass was greater in the treatment sides than in reference sides throughout the season (Fig. 2-2). Average Chl*a* for treatment sides was 35.2 ± 6.8 µg/L (mean ± SE), seven times greater than reference sides ($\bar{x}$=4.96 ± 1.9 µg/L). Differences between treatment and reference pond side Chl*a* concentrations were more dramatic after July 22 (treatment: 55.0 ± 9.2 µg/L; reference: 6.75 ± 2.0 µg/L) compared to prior July 22 (treatment: 7.00 ± 2.0 µg/L; reference: 1.9 ± 0.4 µg/L). In both treatment and reference sides, phytoplankton biomass, as indicated by Chl*a*, increased after July 22.

Qualitative differences in macrophyte growth and biomass were also observed. Greater quantities of submergent and emergent macrophytes occurred in the treatment compared to reference sides. Overall, pond three had the most macrophyte growth and pond two contained the least. Both pond one and two (reference sides) also contained an unidentified orange-brown benthic alga in littoral areas.

Zooplankton composition was similar in all ponds, consisting primarily of calanoid and cyclopoid copepods, various cladocerans and very few rotifers (Appendix I). Zooplankton biomass in all ponds was generally greatest on the first sampling date (June 26), decreasing subsequently and remaining low (Table 2-3). Zooplankton biomass in ponds one and two was not different between the treatment and reference sides throughout the season for each size fraction (Table 2-3).

2.3.2 Stocked Fish

Numbers of stocked fish were not different between treatment and reference pond sides throughout the season (Fig. 2-3). The numbers of stocked fish in all ponds decreased by the end of the season, as expected, due to post-spawning mortality. Most mortality throughout the season occurred in males: 60% of the total males stocked did not survive to the end of the season. Female and juvenile stocked fish mortality were respectively 41% and 43%.

Average lengths of male, female, and juvenile stocked fish measured throughout the season were not significantly different between treatment and reference pond sides for males, females or juveniles (Fig. 2-4).

2.3.3 Egg Stage

In all pond sides, except pond two reference side, spawning commenced mid-June (ca. June 19), a few days after adults were stocked. No eggs were observed prior to June 19 in any pond sides. Spawning coincided with increases in water temperature (Figs. 2-1 and 2-5), peaking in the beginning of July when water temperatures rose to ca. 21°C. When the temperature dropped on July 12 to 17°C, the number of eggs laid in each side also decreased, rising again slightly with the corresponding temperature rise on ca. July 16 back to 21°C. In all pond sides, spawning had ceased by ca. July 30.

Peak hatching also occurred in early July (ca. July 5) for all pond sides, except pond two reference side, with a second increase after mid-July (ca. July 12)(Fig. 2-6). Egg batches, prior to peak hatching (before July 5) generally took 7-8 d to hatch with incubation time decreasing to between 4-5 d after peak hatching. The final date any hatching occurred was August 5.

Pond two's reference side was unique since all eggs were laid and hatched after peak hatching in all other pond sides (after July 5, Figs. 2-5 C and 2-6 C). As a result of the unique spawning and hatching of this pond side, it had the lowest total number of eggs laid and hatched of all pond sides.

The average total number of eggs laid was 1.8 times greater in the treatment ($\bar{x}$=120,137 ± 11,425 (mean ± SE)) than in the reference ($\bar{x}$=65,264 ± 18,423) pond sides. Because overall egg predation and disease was low and unaffected by treatment, the proportion of eggs surviving to hatch was similar (81.1% treatment; 81.2% reference). Consequently, average total number of eggs hatched was also 1.8 times greater in treatment ($\bar{x}$=97,215 ± 8,096) than in reference ($\bar{x}$=55,089 ± 18,997) pond sides. Indeed, in the first half of the spawning season, prior to peak hatch (June 19-July 5), the number of eggs laid (repeated measures ANOVA, $F_{1,2}$=2.07, P=0.29) and hatched ($F_{1,2}$=0.71, P=0.49) was not different between the treatment and reference pond sides, despite the absence of hatching in pond two (reference side). However, in the second half of the spawning season, after peak hatch (July 6 – August 5), more eggs were laid ($F_{1,2}$=66.2, P=0.02) and hatched ($F_{1,2}$=65.8, P=0.02) in the treatment than in reference pond sides.

2.3.4 Age-0 Fish

The densities of age-0 fathead minnows fluctuated throughout the season in the treatment pond sides ranging from 0.46 to 1.75 fish / L (Fig. 2-7). In the reference sides densities of age-0 fish were much lower (0.056 - 0.46 fish / L) compared to treatment pond sides, and decreased consistently after the initial (July 17) sampling date (Fig. 2-

7). No age-0 fish were captured on July 17 for pond two reference side, but subsequent to their first being sampled (July 29), their densities also declined.

Total numbers of age-0 fish surviving to the end of the season were greater in the treatment ($\bar{x}$=11,113 ± 1423) than in reference ($\bar{x}$=1,922 ± 412) pond sides (Fig. 2-8 A). In addition, a consistently greater proportion of age-0 fish survived in the treatment ($\bar{x}$=0.12 ± 0.04) than in the reference ($\bar{x}$=0.04 ± 0.01) pond sides (Fig. 2-8 B).

Generally, length-frequency distributions for age-0 fish sampled in July and August (Fig. 2-9 to 2-11) did not differ between treatment and reference pond sides (K-S tests, P>0.25). However, for certain dates and ponds, length-frequency distributions were different between treatment and reference pond sides (K-S tests, pond one: July 29 (Z=1.38, P=0.04) and August 13 (Z=1.57, P=0.01); pond two: August 13 (Z=1.73, P=0.005); pond three: July 17 (Z=1.67, P=0.008) and July 29 (Z=1.4, P=0.04)).

Final length-frequency distributions were different between treatment and reference sides in all three ponds (K-S tests, pond one: Z=5.22, P<0.001; pond two: Z=1.97, P=0.001; pond three: Z=3.86, P<0.001) (Fig. 2-12). Mean lengths of age-0 fish surviving to the end of the season were greater in the treatment sides of ponds one ($\bar{x}$=19.7 ± 0.23 mm, n=894) and three ($\bar{x}$=22.9 ± 0.28 mm, n=668), compared with their respective reference sides (one: $\bar{x}$=17.6 ± 0.23 mm, n=692; three: $\bar{x}$=21.6 ± 0.32 mm, n=597). Age-0 fish lengths for pond two were not different between treatment ($\bar{x}$=18.1 ± 0.16 mm, n=1070) and reference ($\bar{x}$=18.1 ± 0.18 mm, n=835) pond. The condition of surviving age-0 fish was not consistently different between treatment and reference pond sides (Fig. 2-13).

2.4 DISCUSSION

Natural variation in population size is an important aspect of fish ecology, and its understanding is particularly critical to the management of fish populations (Sissenwine 1984). Although most of this variation can be attributed to survival through the early life-history stages (eggs, larvae, and post-larvae; Miller et al. 1988), the processes and factors that influence survival in these stages are poorly understood. One factor shown to contribute to recruitment in young fish in freshwater ecosystems is nutrient enrichment (Mills 1985; Myers et al. 1996; Tice et al. 1996).

In small-scale aquaculture studies, nutrient addition to small ponds and lakes enhances juvenile fish growth and survival (Fox et al. 1989; Tice et al. 1996; Myers et al. 1996). In such studies, nutrient regimes are manipulated to maximize food abundance for juvenile fish (phytoplankton and small invertebrates). These studies demonstrate a link between nutrient addition and food availability for young fish and, in addition, provide information on the subsequent influence of food availability on juvenile growth and survival. Such studies, however, do not identify the mechanisms influencing juvenile survival, nor do they consider the responses of other life-history stages to nutrient addition and their contributions to recruitment. Further, since these studies are typically conducted at small-scales and at unnaturally high fish densities, results on juvenile growth and survival cannot be automatically extrapolated to natural field conditions.

Several larger, ecosystem-scale studies have investigated the responses of other life-history stages to different nutrient regimes. Initial responses have included faster growth, improved condition, and greater production, abundance, and survival of

juvenile (age-0) fish (Colby et al. 1972; Nakashima and Leggett 1975; Mills 1985; Mills and Chalanchuk 1987). The responses and subsequent contribution of the adult stage (e.g., production of eggs and predation on early-life history stages) to recruitment were generally not included in these studies. Their absence was confounded by the difficulty in sampling the egg stage. Quantification of egg production and mortality has generally relied on indirect (fecundity) estimates (LeBrasseur et al. 1978; Mills 1985). Consequently, estimation of juvenile mortality has been difficult in the absence of an accurate method of quantifying the number of eggs that hatch. In these studies, the specific mechanisms influencing recruitment in fish have been masked by the complexity of the system.

My study on small boreal ponds demonstrated that recruitment of age-0 fathead minnows increased in response to nutrient enrichment. Unlike previous studies, however, I was able to quantify responses and interactions of life-history stages from eggs to adults, to determine the mechanisms influencing this enhanced recruitment of young. The details of these responses are discussed below as they relate to each of the major objectives set out in the introduction of this chapter.

2.4.1 Biotic Environment (Food Availability)

In response to nutrient addition, average algal biomass increased in treatment pond sides 7x above levels in reference sides. Nevertheless, concentrations for all pond sides fell within the range commonly found in lakes and ponds of central Alberta (1.9 µg/L – 100.5 µg/L; Prepas and Trimbee 1988). Although phytoplankton biomass was greatest in the second half of the season (after July 22) in both sides of each

experimental pond, maximum differences between treatment and reference sides also occurred in the second half of the season.

Phytoplankton, filamentous algae, and other plant material, can be important food sources for all life-history stages of the fathead minnow, including smaller juveniles (see Held and Peterka 1974, Tallman et al. 1984, Shaw et al. 1995). Although several studies suggest that zooplankton and insect larvae are the main food source for fathead minnows (Held and Peterka 1974; Price et al. 1991), this species has been referred to as the "temperate generalist" (see Tallman et al. 1984). Fathead minnows appear to be flexible in their choice of temporarily available food items that can vary among habitats, community structures, and geographic locations. Therefore, in my experimental ponds, seasonal changes in phytoplankton biomass within and between pond sides may have influenced food availability for fathead minnows.

No differences in zooplankton biomass were observed throughout the season between the treatment and reference pond sides. In freshwater systems, bottom-up regulation of biomass is generally strongest near the bottom of the food web and conversely, top-down regulation of biomass is generally strongest near the top of the food web (McQueen et al. 1986). Several studies have shown specifically that high fish densities can decrease biomass of zooplankton (Galbraith 1967; Zaret 1972; Spencer and King 1984; Diana et al. 1991; Tonn et al. 1994). Since higher densities of age-0 fish occurred in the treatment sides, similar zooplankton biomass between treatment and reference pond sides may have reflected increased zooplankton production in treatment sides. Therefore, increased zooplankton production in the treatment pond sides may have, in addition to phytoplankton, increased food supply for the fish.

2.4.2 Adult and Egg Stage

Very few studies have documented the responses of older fish to nutrient enrichment (Mills 1984; Mills and Chalanchuk 1987). Instead, most have focused on the responses of younger fish (<1 y) (e.g., LeBrasseur et al 1978; Tice et al. 1996; Fox et al. 1989), since processes acting on these stages directly contribute most to recruitment in fish populations (Sissenwine 1984; Miller et al. 1988). Despite the potential influence of adult stages on both the numbers of eggs produced (Wootton 1977, 1990) and predation on the early life-history stages (Sissenwine 1984; Bailey and Houde 1989; Crowder et al. 1992; Vandenbos 1996), mechanistic studies on recruitment rarely include adult stages (Rice et al. 1987; Crowder et al. 1992; Cargnelli and Gross 1996). Understanding the responses of adults to increased food and the subsequent contributions of adults to the mechanisms that influence recruitment (production of eggs, and the mortality of the egg, larval, and juvenile stages) is necessary to predict population size fluctuations.

In my study, mortality of stocked fish was not different between treatment and reference pond sides despite observed differences in food availability. These results are similar to other studies where nutrient addition has not affected survival of older fish (> age-1) (Nakashima and Leggett 1975; Mills 1984; Mills and Chalanchuk 1987). Most mortality in adult fathead minnows occurs after spawning, associated with stresses during the spawning period (Markus 1934; Vandenbos 1996). In my study, post-spawning mortality was high (40 –60%) and was not influenced by differences in food availability between the treatment and reference pond sides.

Lengths of stocked fish were also not different between treatment and reference pond sides. For adult fatheads the spawning period is a period of energy loss (Tallman

et al. 1984). At this time, surplus and stored energy is allocated in males to the development of secondary sexual characteristics and gonads, and to reproductive behavior (guarding, cleaning, and aerating eggs) (Wootton 1990). Similarly, in females, most of their surplus energy is allocated to gonad rather than somatic growth during the spawning season. Since fatheads are iteroparous (they can survive to breed several times), up to 60% of their stored body energy may have been used for reproduction (Diana 1995). Female sticklebacks (*Gasterosteus aculeatus*) require a high ration size to prevent weight loss during spawning (Wootton 1977). Further, growth in the nine-spined stickleback (*Pungitius pungitius*) does not occur until after spawning (Griswold and Smith 1973). Therefore, despite differences in food availability between the treatment and reference sides, the lack of differences in growth of stocked fish may be attributed to the short period of time during the experiment these fish were not spawning and, hence, able to allocate energy to somatic growth.

Although juveniles did not have secondary sexual characteristics at the time of stocking, shortly after, secondary sexual characteristics began to develop on both males and females, and females became gravid. Therefore, juveniles may also have allocated surplus food energy to the development of secondary sexual characteristics and gonads and to reproductive behavior rather than somatic growth, resulting in non-significant differences in lengths between treatment and reference sides.

Even though the total number of eggs laid in reference sides was greater than observed in previous years for fathead minnows stocked at similar densities (Vandenbos 1996), production of eggs increased more in treatment sides with nutrient addition. The number of eggs laid differed between treatment and reference pond sides only in the

second half of the spawning season (July 6 – August 5), but this was consistent with the timing of the differences in food availability (as indicated by algal biomass) between pond sides.

Food supply may affect egg production in female fathead minnows in several ways. Ration size for multi-batch spawning fish can a) directly affect the number of times an individual female can spawn, b) inversely affect the time between successive spawnings, and c) directly affect batch fecundity (Wootton 1977, 1990; Reznick and Yang 1993). Changes in each, or all, of these factors could have influenced the total number of eggs laid throughout the season.

In pond two's reference side little or no egg production occurred in the first part of the spawning season (prior to July 6). The few eggs that were observed early in the spawning season were not viable, and disintegrated upon contact. Interestingly, pond two had the lowest algal biomass (Chl*a*) of all ponds throughout the season. Therefore, it is possible that the initial amount of available food was so poor that females either resorbed many of their yolked oocytes or failed to produce vitellogenic eggs (Wootton 1990). Much remains to be known about the effects of environmental factors, such as food availability, on batch and breeding-season fecundity of multi-batch-spawning fishes (Wootton 1977, 1990), yet such knowledge is essential to predict fish population size fluctuations in response to nutrient enrichment.

Addition of nutrients did not influence overall cannibalism of eggs. Mortality of eggs, through to hatch, was relatively low (ca. 18%) in both treatment and reference pond sides. This amount is consistent with egg mortality observed in a previous study, conducted in the same ponds at similar densities of stocked fish (Vandenbos 1996).

However, I had expected lower egg mortality in the treatment pond sides. Greater quantities of alternative food to potential cannibals could decrease cannibalism (Margulies 1990; Gotceitas and Brown 1993). In addition, improved male condition, in response to nutrient addition, should improve their ability to guard their eggs. Perhaps densities of fish were too low to see an influence of food on these proposed mechanisms. Vandenbos (1996) hypothesized that school size may be the main factor influencing cannibalism of eggs, since males should have greater difficulties defending nests against higher densities of fish. It is possible, but remains untested, that in high-density conditions, food availability could influence cannibalism.

2.4.3 Age-0 Fish

Densities of age-0 fish were greater in nutrient enhanced pond sides compared to reference pond sides throughout the season (July – September 1996). Survival of age-0 fish to the end of the season (September 8) was also greater in the treatment pond sides. Therefore, in addition to an increase in the number of eggs produced, nutrient enrichment also appeared to influence the main mortality mechanisms (predation and starvation) acting on age-0 fathead minnows.

Increased survival of age-0 fish due to nutrient enhancement has been observed in both small-scale aquaculture studies (Fox et al. 1989; Tice et al. 1996; Myers et al. 1996) and large-scale eutrophication studies (Colby et al. 1972; Mills 1985; Mills and Chalanchuk 1987). This response is generally attributed to bottom-up processes that increase the main sources of food (phytoplankton and zooplankton) for young fish (Colby et al. 1972; Schindler et al. 1973; LeBrasseur et al. 1978; Stockner and Shortreed 1985). In response to variation in food supplies, changes in fish survival are

expected to occur particularly in young fish (<1 y old) since this is when the greatest variation in mortality occurs (Sissenwine 1984).

Greater alternative food availability in the treatment pond sides may have increased survival of age-0 fatheads through the reduction of predation on these fish. As previously mentioned, since densities of stocked fish in the ponds were relatively low and male parents guard their eggs, males might have been equally successful at defending their nests regardless of the influence of alternative food availability on the intensity of nest raiding by potential predators. However, since male parents do not guard age-0 fish, the intensity of predation on these stages may have been lower in the treatment pond sides due to the increase in alternative food availability. When alternative prey are present in large numbers, they can interfere with the foraging on young fish by predators (Margulies 1990). Factors such as size, movement, and evasiveness of alternative prey may also influence their selection over young fish by the predators, decreasing overall mortality of young fish (Gotceitas and Brown 1993).

Food availability can also influence survival in young fish through its influence on growth rates. Since adult fatheads are gape-limited predators, with the largest adults (>70 mm) restricted to age-0 fatheads <19 mm (Vandenbos 1996), faster growing age-0 fish should experience less mortality due to predation. Faster growth should also improve a young fish's ability to avoid predators and capture prey items, since larger fish have improved visual acuity (Fernald 1988) and swim speeds (Yates 1983). The faster a fish grows, the less time it will spend in a smaller more vulnerable size and, as a result, mortality due to both predation and starvation should be reduced.

In my study, size-distributions of age-0 fish at the end of the season were skewed to larger sizes in the treatment sides of all ponds. This suggests that growth rates were greater in the treatment than in the reference sides. A separate enclosure study I conducted also reported faster growth rates in response to increased food supplies for age-0 fathead minnows (Kiesling, unpublished data). Therefore, increased growth of treatment fish, due to an increase in available food, should have contributed to the increased numbers of age-0 fish that survived in the treatment sides than in the reference sides.

In pond two, since most fish hatched after July 5 in the reference side, whereas peak hatching in the treatment sides occurred prior to July 5, reference fish should have had, on average, a shorter time to grow. In the reference sides, food availability was low (it was actually lowest in this pond side compared to all other pond sides) and survival of age-0 fish was lower than in the treatment pond side. This suggests that in the reference pond side, slower growing, smaller fish, might have experienced greater mortality compared to fish in the treatment pond sides. As a result, fish that survived to the end of the season in the reference pond side would have been the faster growing individuals compared to fish in the treatment pond sides.

Size-selective mortality might also have contributed to the final size-distributions of reference fish in ponds one and three. Post and Prankevicius (1987) reported size-selective mortality only in slower growing age-0 yellow perch (*Perca flavescens*), as they spent more time in a size more vulnerable to both predation and starvation. Therefore, survivors in a generally slower growing cohort (reference sides) should be, on average, the larger, faster growing individuals.

Body size at the end of the growing season has important implications for survival over the winter period since survival of age-0 fish is directly related to body size (Post and Evans 1989; Smith and Griffith 1994; Kiesling, unpublished data). Over the winter period, food availability is low and feeding activity is slowed by cold temperatures; therefore, to survive, fish must rely on stored energy. Since smaller individuals have lower energy reserves and higher mass-specific standard metabolic rates (Brett and Groves 1979; Shuter and Post 1990), these fish would be expected to deplete their energy reserves quicker than larger fish. Therefore, nutrient enrichment of aquatic ecosystems, by increasing growth of age-0 fish during their first summer, should enhance survival over the winter period, provided increased productivity in the summer growing season does not deplete dissolved oxygen in the winter period.

2.4.4 Population Response to Climate and Land-Use Change

Associated with a predicted increase in precipitation due to climate change, particularly in northern latitudes (Hansen et al. 1991), runoff and nutrient loads to aquatic ecosystems should also increase (Waggoner 1990; Carpenter et al. 1992). In northern Alberta, an increase in nutrient loads may also result from increased logging and agriculture (Schlesinger 1997; Sharpley and Smith 1993).

If increased nutrient loads increases food availability, my results indicate that populations of fathead minnows will respond by laying more eggs and age-0 fish should experience increased growth and decreased mortality in their first growing season. As a result, greater numbers and larger sizes of age-0 fish should survive to the end of the summer. Since larger fish are less susceptible to overwinter starvation, more fish should also survive to the end of the winter period, provided that oxygen depletion does

not become severe. Therefore, an overall increase in productivity in aquatic ecosystems should increase fathead minnow population sizes in northern Alberta.

2.4.5 Future Studies

My pond study represents one analysis of the effects of nutrient enrichment on recruitment of age-0 fish. Smaller-scale enclosure studies on newly hatched fathead minnows should be conducted to determine how variation in food availability influences growth rates and subsequent mortality due to predation and starvation (as proposed in Chapter Four). Such smaller-scale experiments can also facilitate the study of a broader food gradient, representing the full range of food availability experienced in nature, on growth and mortality of age-0 fish. Small-scale mechanistic studies should also determine how the intensity of cannibalism by adult fathead minnows varies with the abundance of alternative foods. The influence of increased ration size on the number of eggs laid, the time interval between spawnings and the number of times an individual fish can spawn within a season should be evaluated (Wootton 1977, 1990).

My pond study should also be repeated with a greater number of replicates to provide a more rigorous examination of the effects of nutrient enrichment on mortality of age-0 fathead minnows. Further, the pond study should be conducted for a longer time to determine if the observations in the first year of nutrient enhancement apply in subsequent years. Longer-term studies are also necessary to determine the effects of increased nutrient loads on overwinter mortality in fish. For fish populations in northern ponds and lakes, the winter period can be a significant determinant of recruitment of young fish into the adult population both because of energetic reasons (Shuter and Post 1990) and as a result of dissolved oxygen depletion.

The combination of small-scale mechanistic studies, larger-scale studies on ponds, and studies on natural populations can help to describe quantitatively the factors and mechanisms underlying recruitment variation in fishes. My pond study results, combined with the studies just proposed, will be particularly important if we are to understand and accurately predict the responses of natural fish populations to increasing climate and land-use changes.

Table 2-1. Mean (± SE) nutrient concentrations for three experimental ponds (treatment and reference sides), measured from June – August, 1996 (sampled after the initial June 13 nutrient addition). *F*-value and significant differences for each variable between treatment and reference sides are indicated, determined from repeated measures ANOVA. Data for total phosphorus and total nitrogen do not include the July 24 sampling date.

Variable	Mean (SE) µg/L		$F_{1,2}$
	Treatment	Reference	
Total phosphorus (n=9)[1]	47.5 (4.6)	21.2 (2.0)	15.4
Total dissolved phosphorus (n=5)[1]	20.0 (2.3)	7.04 (0.3)	32.9*
Total nitrogen (n=2)[1]	864 (142)	521 (32.7)	7.29
Ammonium (n=8)[1]	65.3 (15)	10.5 (1.4)	4.70
Nitrates + nitrites (n=8)[1]	277 (37)	4.20 (0.6)	19.3*

[1]sample size per pond side
*$0.01 < P \leq 0.05$

Table 2-2. Average dissolved oxygen concentrations and pH for three experimental ponds (treatment and reference sides) measured from June – August, 1996. *F*-values for the differences between treatment and reference pond side variables are indicated, determined from repeated measures ANOVA. Note: none of these differences were significant.

Variable	Mean (SE)		$F_{1,2}$
	Treatment	Reference	
Dissolved Oxygen (n=9)[1] (mgO_2/L)	9.4 (0.58)	6.6 (0.66)	4.28
pH at surface (n=6)[1]	8.8 (0.10)	8.0 (0.08)	7.98
pH at bottom (n=6)[1]	8.8 (0.12)	7.9 (0.09)	5.17

[1]sample size per pond side

Table 2-3. Zooplankton dry biomass (μg/L) for ponds one and two, treatment (T) and reference (R) pond sides, from June – August, 1996, with inshore and offshore sites pooled. Zooplankton biomass was not significantly different between treatment and reference sides throughout the season for each size fraction for pond one and two (repeated measures ANOVA, small: $F_{1,1}$=4.64, P=0.28; medium: $F_{1,1}$=7.79, P=0.219; large: $F_{1,1}$=0.66, P=0.57).

		Mean					
		June 26		July 22		August 19	
Pond Number	Size	T	R	T	R	T	R
Pond One	Small[a]	0.047	0.055	0.025	0.004	0.025	0.004
	Medium[b]	0.022	0.018	0.006	0.005	0.006	0.005
	Large[c]	0.014	0.015	0.011	0.008	0.011	0.008
	TOTAL	0.083	0.088	0.042	0.017	0.042	0.017
Pond Two	Small[a]	0.022	0.008	0.004	0.006	0.006	0.006
	Medium[b]	0.042	0.024	0.002	0.017	0.017	0.017
	Large[c]	0.042	0.058	0.020	0.044	0.022	0.044
	TOTAL	0.106	0.091	0.026	0.066	0.044	0.066

[a] <153 μm
[b] 153-500 μm
[c] >500 μm

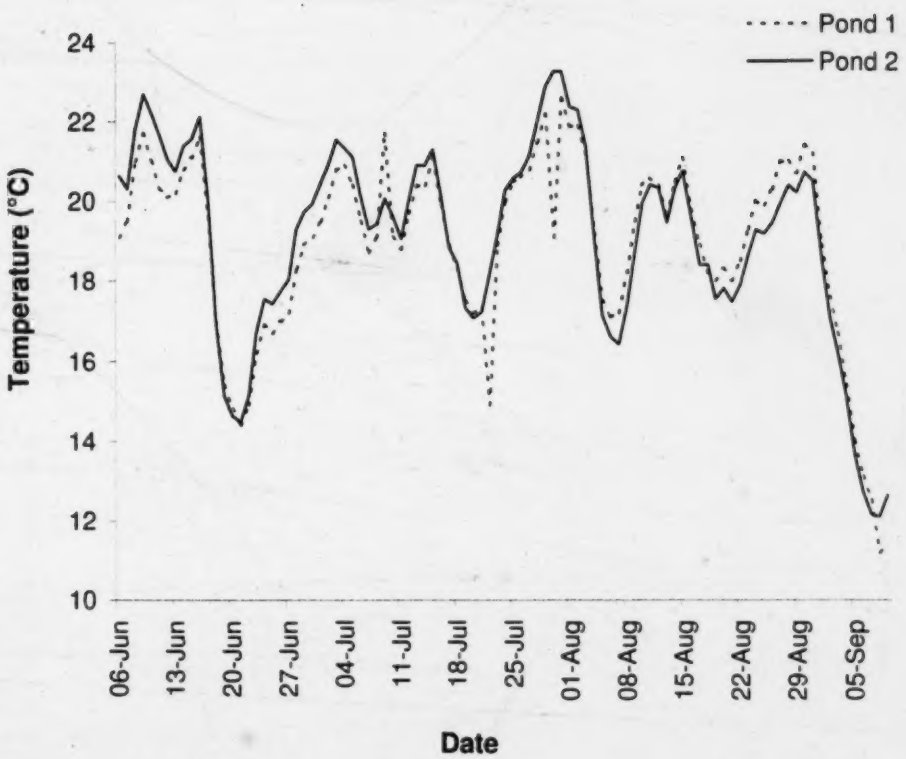

Fig. 2-1. Mid-depth (0.75 m) water temperature for pond one and two (treatment sides), from June to September, 1996. Temperatures were not different between pond one and two (paired *t*-test, t=-0.68, df=109, P=0.5).

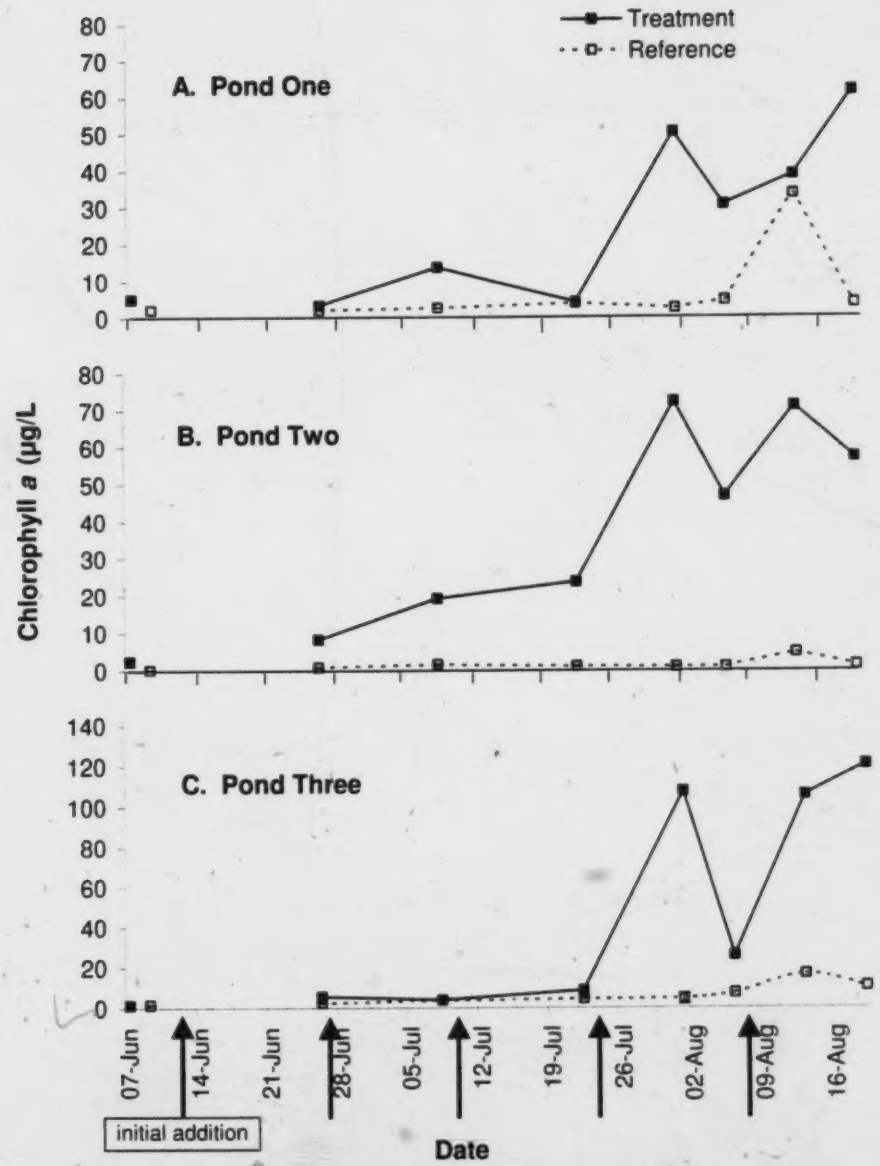

Fig. 2-2. Chlorophyll *a* measured for three experimental ponds (A – C) (treatment and reference sides) from June – August, 1996. Arrows represent nutrient addition dates. Chlorophyll a was greater in the treatment than in reference sides throughout the season (repeated measures ANOVA, $F_{1,2}$=22.2, P=0.04).

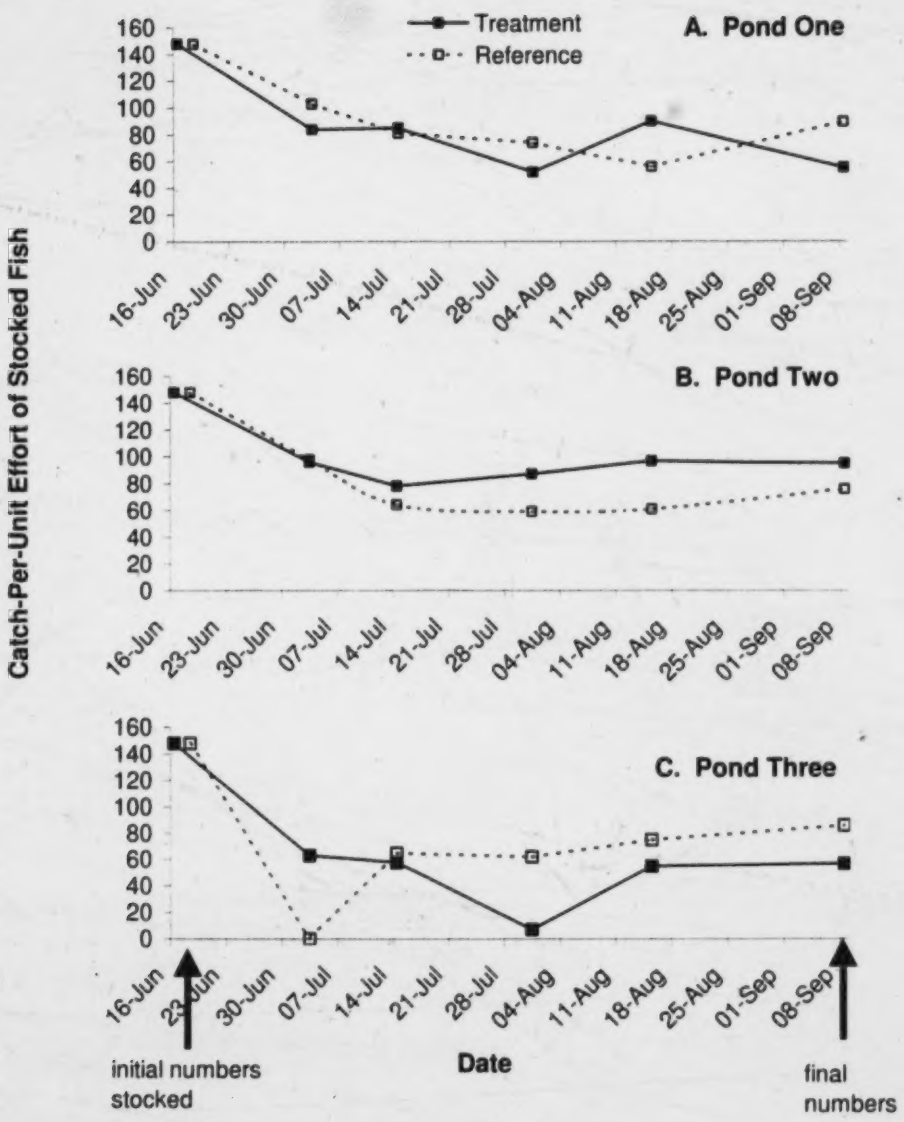

Fig. 2-3. Catch-per-unit-effort (number of fish / 6 traps) for A. pond one, B. pond two, and C. pond three stocked fathead minnows in both treatment and reference sides from June to September, 1996. June 16 are initial numbers of fish stocked and September 8 are final total numbers of fish seined, not CPUE. The numbers of stocked fish were not different between treatment and reference pond sides throughout the season (repeated measures ANOVA, $F_{1,2}$=0.005, P=0.95).

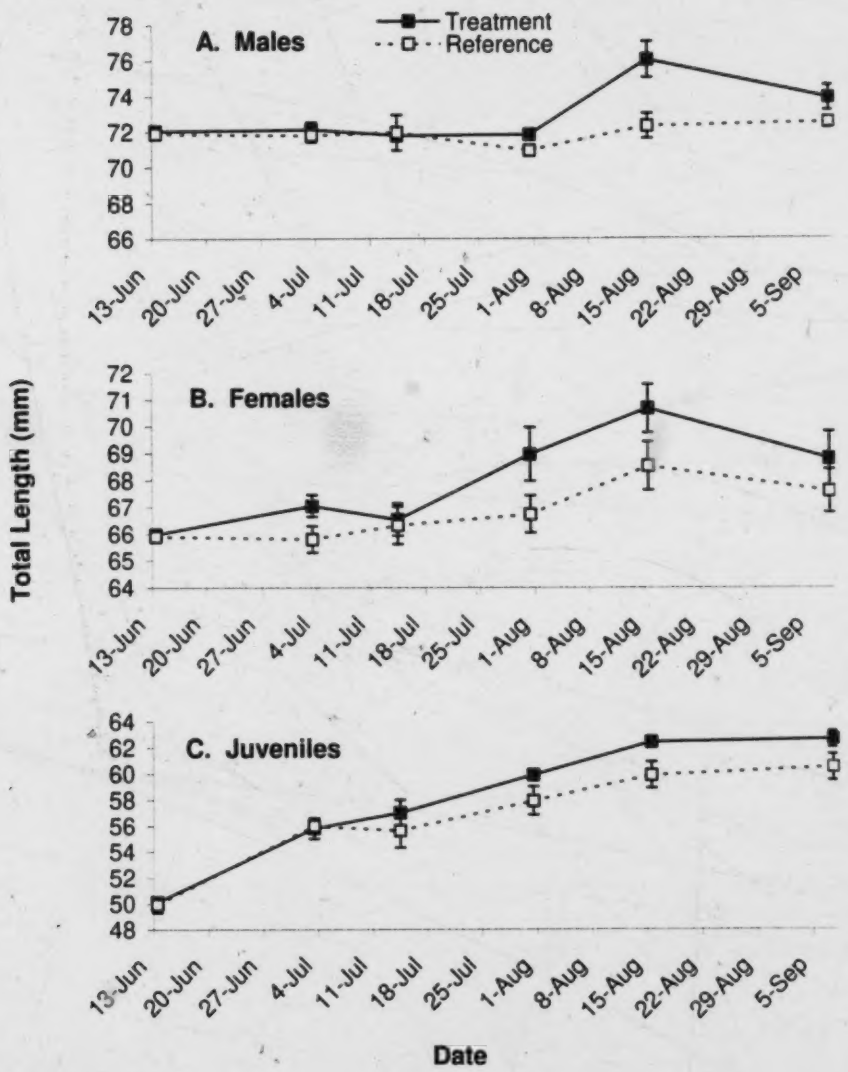

Fig. 2-4. Average total lengths for A. male, B. female, and C. juvenile stocked fathead minnows in both treatment and reference pond sides from three experimental ponds from June to September, 1996. Bars represent ± one SE, N=3. Lengths were not different between treatment and reference sides throughout the season for males ($F_{1,2}$=7.79, P=0.11), females ($F_{1,2}$=5.53, P=0.14) or juveniles ($F_{1,2}$=2.21, P=0.28), repeated measures ANOVA.

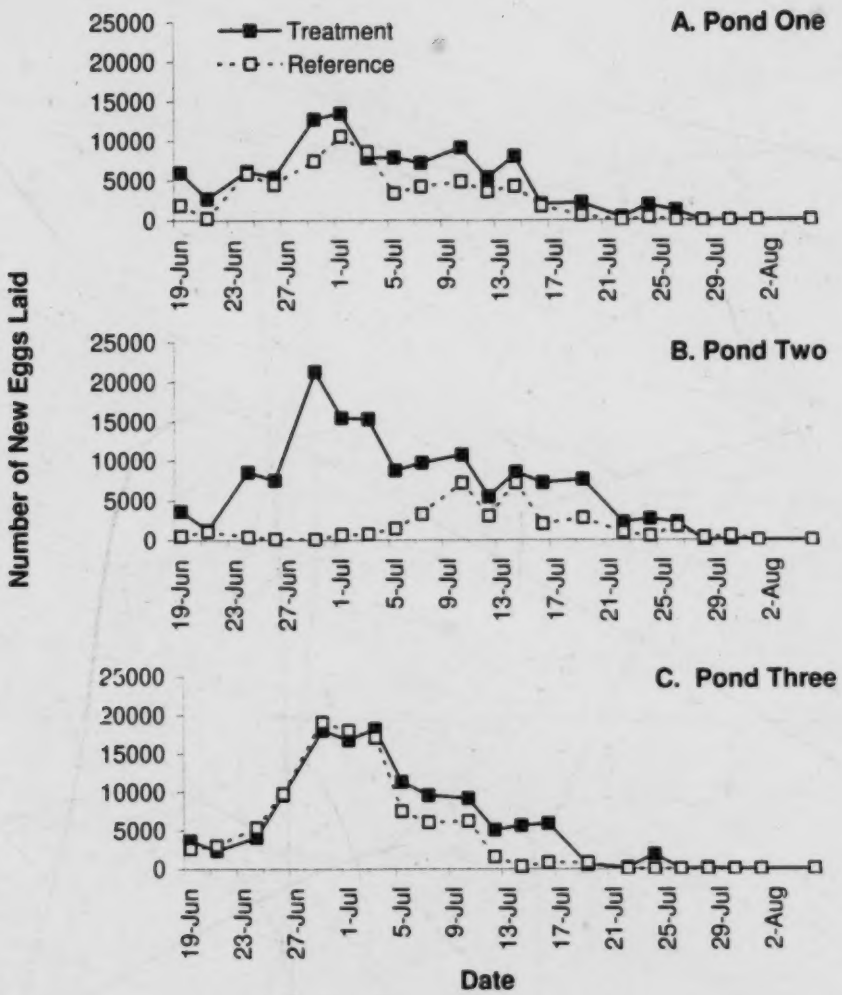

Fig. 2-5. Number of new fathead minnow eggs laid every second day for treatment and reference ponds sides of A. pond one, B. pond two, C. pond three for the 1996 spawning season.

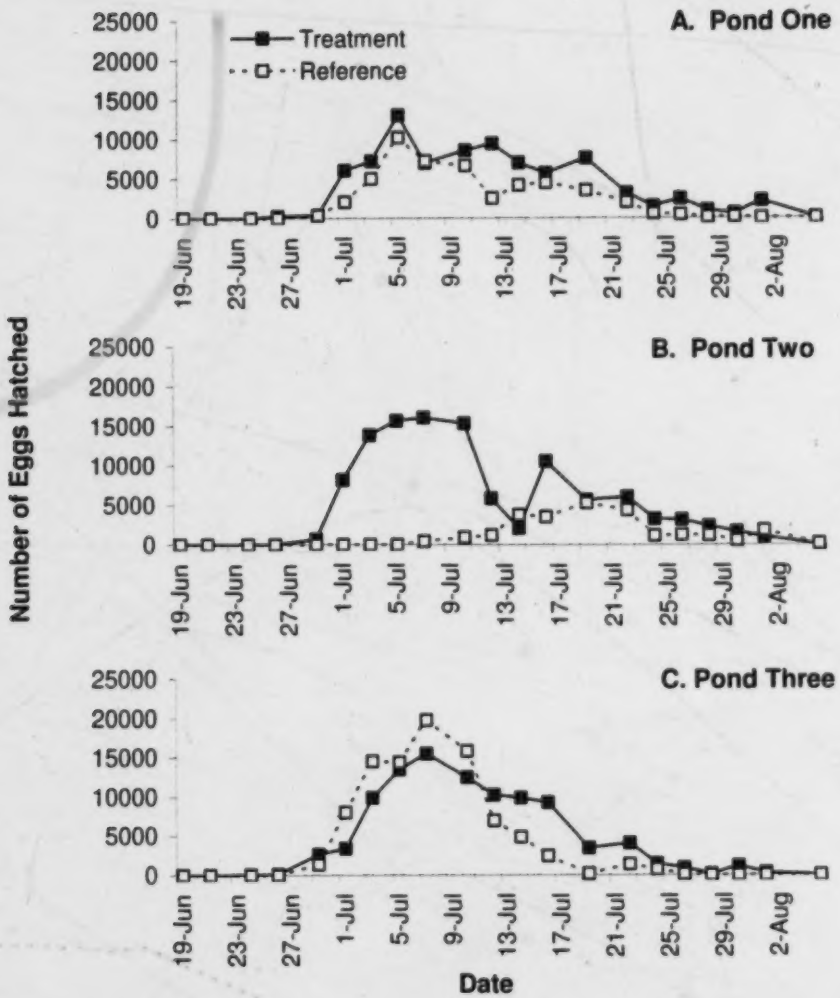

Fig. 2-6. Number of fathead minnow eggs hatched every second day for treatment and reference ponds sides of A. pond one, B. pond two, C. pond three for the 1996 spawning season.

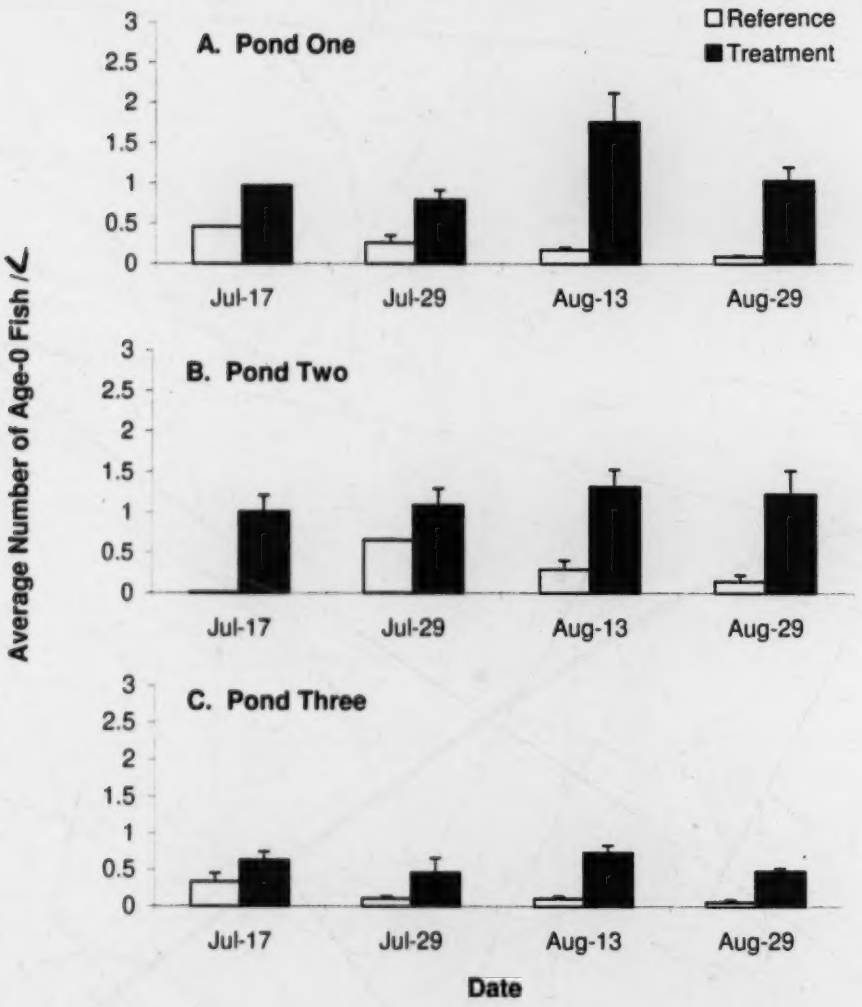

Fig. 2-7. Average number of age-0 fathead minnows per litre for pond one – three (A – C) treatment and reference sides, on four dates from June to September, 1996. Bars represent one SE. Age-0 fish densities were greater in the treatment than reference sides throughout the season (repeated measures ANOVA, $F_{1,2}$=22.7, P=0.04)

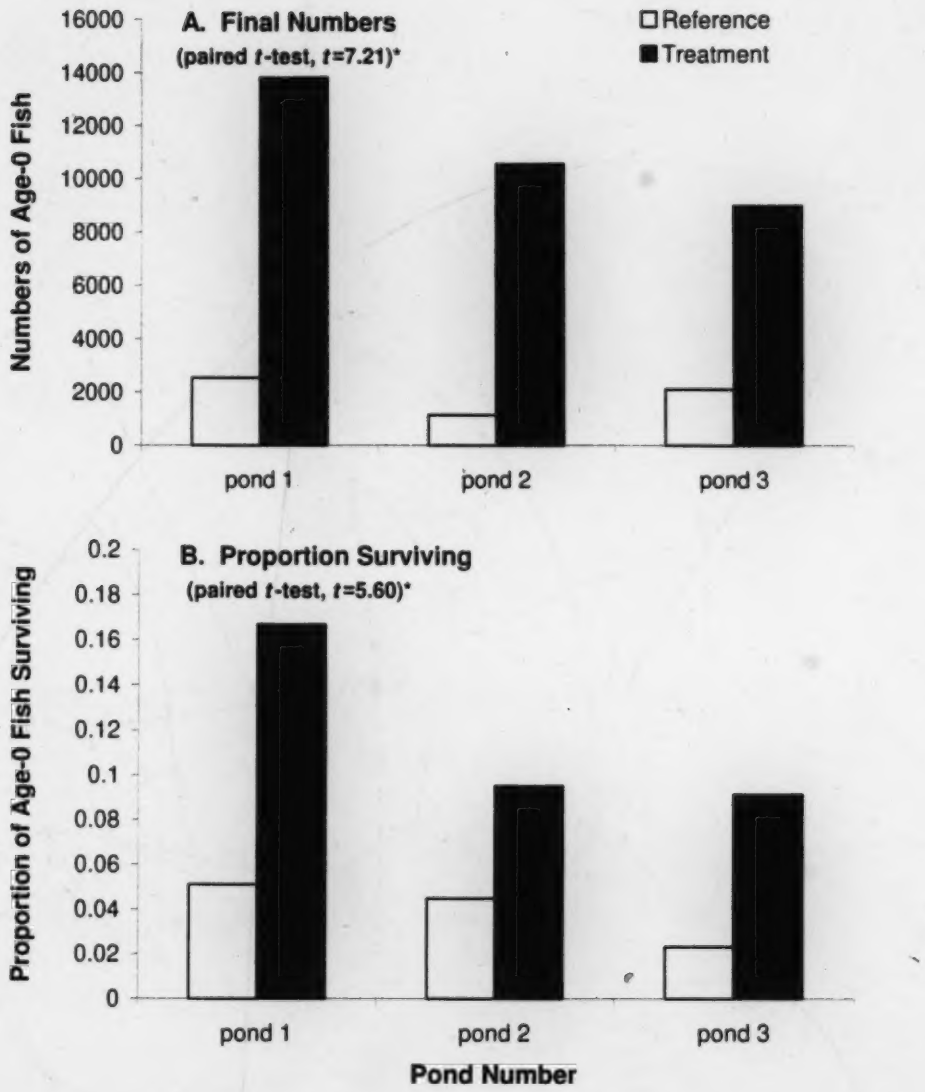

Fig. 2-8. A) Total number of age-0 fathead minnows that survived to the end of the season (September 8, 1996) for each of three ponds' treatment and reference side. B) The proportion (total number surviving / total number initially produced (hatched)) of age-0 fathead minnows that survived to the end of the first season for each pond's treatment and reference side. *$0.01 < P \leq 0.05$.

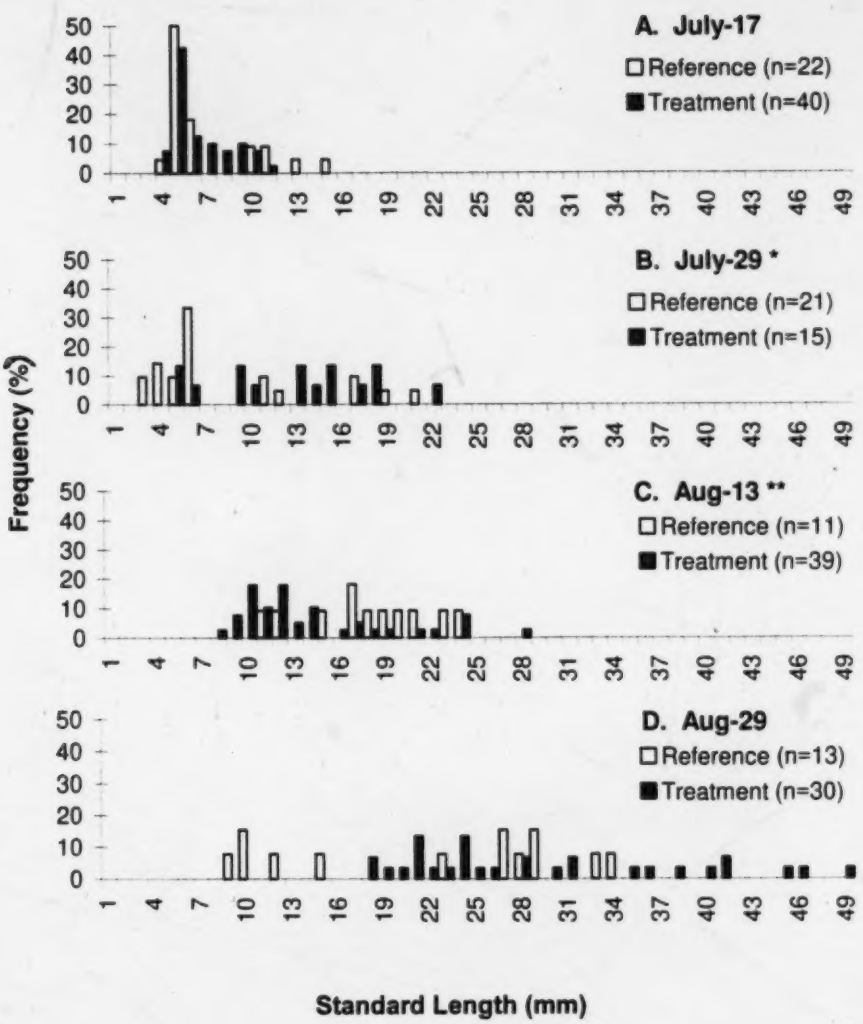

Fig. 2-9. Length-frequency distributions for age-0 fathead minnows in pond one treatment and reference sides on four sampling dates (A – D) during July – August, 1996. Kolmogorov-Smirnov two-sample test (*$0.01 < P \leq 0.05$, **$0.001 < P \leq 0.01$).

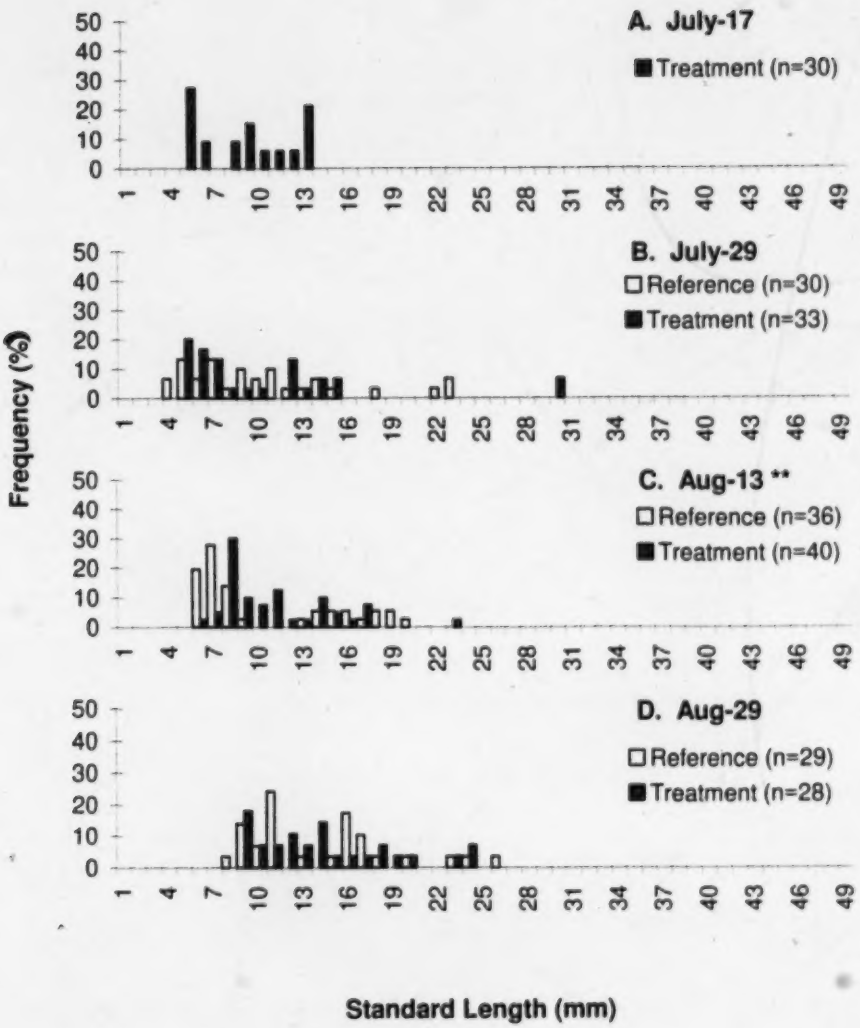

Fig. 2-10. Length-frequency distributions for age-0 fathead minnows in pond two treatment and reference sides on four sampling dates (A – D) during July – August, 1996. Kolmogorov-Smirnov two-sample test (**$0.001 < P \leq 0.01$).

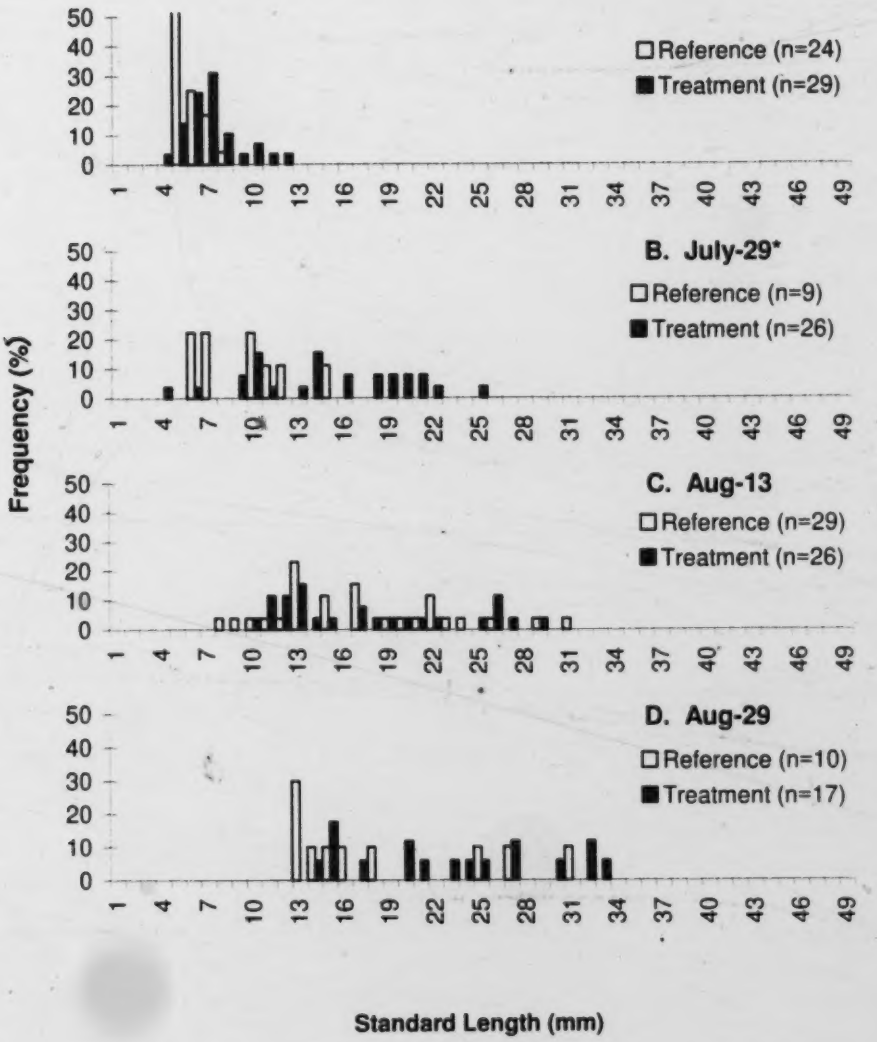

Fig. 2-11. Length-frequency distributions for age-0 fathead minnows in pond three treatment and reference sides on four sampling dates (A – D) during July – August, 1996. Komogorov-Smirnov two-sample test (*$0.01<P\leq0.05$, **$0.001< P\leq0.01$).

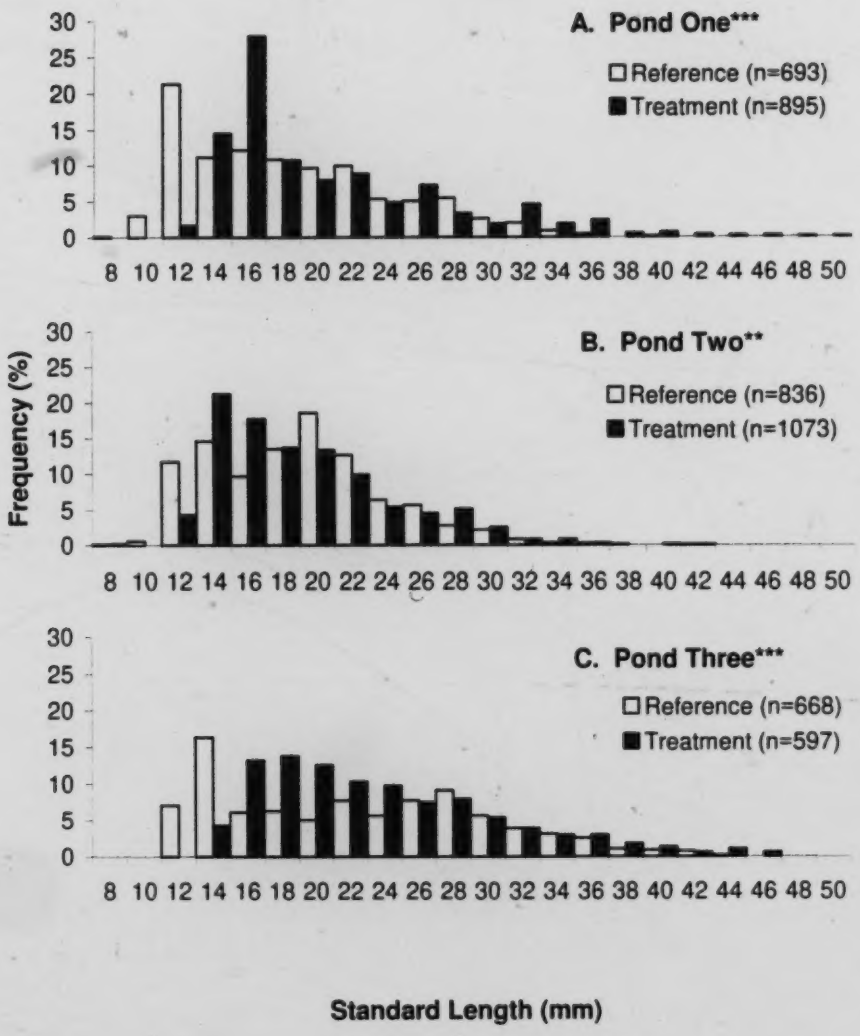
A. Pond One***
Reference (n=693)
Treatment (n=895)
B. Pond Two**
Reference (n=836)
Treatment (n=1073)
C. Pond Three***
Reference (n=668)
Treatment (n=597)
Frequency (%)
0 5 10 15 20 25 30
8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
Standard Length (mm)

Fig. 2-12. Length-frequency distributions for age-0 fish that survived to the end of the first season, prior to ice-up (September 8, 1996) in treatment and reference pond sides for three experimental ponds. Komogorov-Smirnov two-sample test (**$0.001<P\leq 0.01$, ***$P\leq 0.001$).

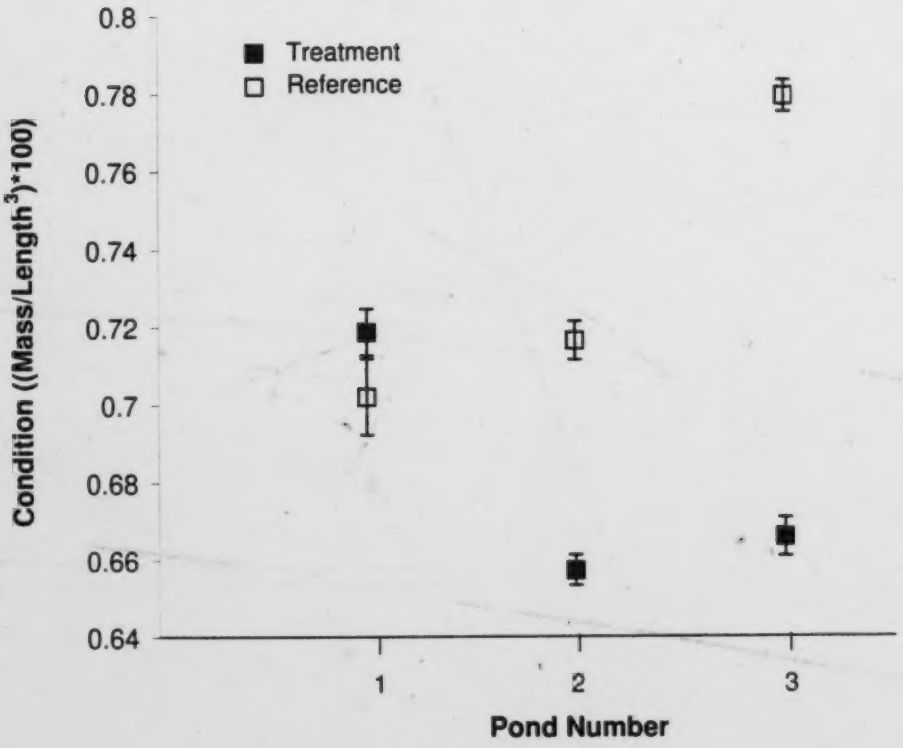

Fig. 2-13. Average condition index (± SE) for age-0 fathead minnows that survived to the end of their first growing season (September 8, 1996) in treatment and reference sides for three experimental ponds. Condition was not different between treatment and reference sides (paired *t*-test, t=-1.32 df=2, P=0.32).

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3. AN EXPERIMENTAL INVESTIGATION OF SIZE- AND HATCH-DATE-SELECTIVE MORTALITY IN AGE-0 FATHEAD MINNOWS (*PIMEPHALES PROMELAS*) USING OTOLITH MICROSTRUCTURAL ANALYSIS

3.1 INTRODUCTION

Fluctuations in fish population size can be largely attributed to processes that act in the early life-history stages (egg, larval, post-larval stages) (Sissenwine 1984; Bailey and Houde 1989). Two key sources of mortality for these stages that have been hypothesized to regulate recruitment are starvation (Hjort 1914) and predation (Sissenwine 1984; Bailey and Houde 1989). The intensity of these sources of mortality can be influenced by various factors, such as food availability, water temperature, etc. Ultimately, quantification of these factors and their effects on the intensity of mortality mechanisms in natural fish populations should assist in the understanding and prediction of fish population dynamics. However, due to the size and complexity of most aquatic ecosystems, current research programs are not able to accomplish this task.

Recently, a shift of the study unit from the population to the individual has advanced our understanding of recruitment dynamics (Post and Prankevicius 1987; Rice et al. 1987; Crowder et al. 1992; Cargnelli and Gross 1996; Gleason and Bengtson 1996). Since most fish die within the first few weeks of life, the individuals that survive may be unique with respect to characteristics that facilitated survival during this early period. A fish's hatch date, growth rate, and body size are all characteristics that can influence its susceptibility to mortality (Post and Prankevicius 1987; Rice et al. 1987; Miller et al. 1988). By comparing characteristics of surviving fish with those from fish

of the same cohort sampled at earlier dates, characteristics that differentiate survivors may reveal how sources of mortality act.

The discovery of daily growth increments on otoliths of young fish (Panella 1971; 1974) has been critical to the development of individual-based approaches in recruitment research. Since this discovery, otoliths have been used to determine the hatch date (Rice et al. 1987; Cargnelli and Gross 1996; McGovern and Olney 1996; Bulak et al. 1997), body size (Post and Prankevicius 1987; Gleason and Bengston 1996), and growth histories (Deegan and Thompson 1987; Penney and Evans 1985) of surviving fish in a population. Growth increments can therefore be used to compare these characteristics between surviving fish and all members of the cohort alive at earlier dates. If surviving fish are unique with respect to these characteristics and if the factors that influence the intensity of mortality are identified, then this approach can provide a quantitative description of how these factors influence recruitment in fish.

Body size and growth rate in young fish are characteristics that can be used to quantify the mechanisms underlying recruitment variation since both starvation and predation are size-dependent processes (Miller et al. 1988). Larger body sizes can improve a fish's ability to capture food and avoid predators due to the associated increases in reactive distances (visual acuity) (Fernald 1988) and swimming speed (Yates 1983). Further, faster growth rates should reduce the time a fish spends in smaller, more vulnerable sizes. As a result, larger body sizes and faster growth rates should decrease the intensity of size-selective mortality acting on young fish.

The intensity of size-selective mortality in young fish can vary among aquatic ecosystems, years, and seasons due to changes in factors (e.g., food availability and

temperature) that influence growth rates; faster growth rates should decrease size-selective mortality. For example, comparing two populations of yellow perch (*Perca flavescens*), Post and Prankevicus (1987) found size-selective mortality to be most pronounced in the slower growing population. Further, if mortality is particularly intense at certain times within a growing season, very few fish from this period survive (Crecco and Savoy 1985; Rice et al. 1987; McGovern and Olney 1996; Bulak et al. 1997). As a result, earlier or later hatching fish may be over-represented among survivors, resulting in mortality that is selective with respect to hatch-date.

One factor that can influence growth, and hence survival, in young fish is the availability of food (Frank and Leggett 1982; Diana 1995; Michaletz 1997). The main food sources for young fish, including phytoplankton and zooplankton, can fluctuate within a season and among years due to changes in biogeochemical cycles within aquatic ecosystems (Schlesinger 1997). However, a more significant influence on food availability is external nutrient inputs to aquatic ecosystems. Experimental manipulation of nutrient loads to aquatic ecosystems have significantly altered phytoplankton and zooplankton biomass (Colby et al. 1972; Schindler et al. 1973; LeBrasseur et al. 1978; Stockner and Shortreed 1985). Several factors can increase nutrient loading into aquatic ecosystems, including global warming and land-use practices.

A global warming of 2 - 4°C has been predicted for the coming decades, based on observations of increased concentrations of carbon dioxide gas in the Earth's atmosphere resulting primarily from the burning of fossil fuels (Hansen et al. 1984). The most significant increases in mean seasonal temperatures have been observed

recently in higher latitudes of North America and Eurasia (Hansen et al. 1996). In response to global warming, climate models predict an increase in intense moist-convective events such as thunderstorms (Hansen et al. 1991), which should increase above ground runoff and nutrient inputs to lakes (Waggoner 1990).

Increased transport of nitrogen and phosphorus into aquatic ecosystems can also occur as a result of human land-use practices, particularly following land-clearing activities, such as logging and agriculture. Removal of vegetation from a terrestrial ecosystem should increase soil erosion and runoff into aquatic ecosystems due to the reduction of the vegetation canopy, soil surface roughness and plant transpiration (Schlesinger 1997). Since most nutrients are retained in terrestrial ecosystems by vegetation, its removal should increase the amount of nutrients in runoff water. Fertilizer use in agriculture can also increase the concentrations of nitrogen and phosphorus in runoff waters (Sharpley and Smith 1992). As a result, aquatic systems located within catchments subjected to forest harvesting and agricultural practices should experience an increase in nutrient loads. Therefore, via impacts on food availability and growth, both climate warming and land-use changes may alter the intensity of size-selective mortality on young fish, ultimately altering rates of recruitment and population dynamics.

Understanding the impacts of nutrient loading is particularly important for populations of fathead minnows (*Pimephales promelas*) in northern Alberta. In this region increased runoff resulting from global climate change represents a potential source of enhanced nutrient supply to aquatic ecosystems. In addition, there is concern that rapid increases in forestry activities and agriculture will also increase nutrient

inputs. Since fathead minnow populations in Alberta are at the northern edge of their distribution (Scott and Crossman 1973), the recruitment rate is likely at the low extreme for this species (Shuter and Post 1990). Therefore, through its influence on food availability changes in nutrient loads may influence recruitment in these populations.

In a pond study, increased nutrient loads significantly enhanced survival of age-0 fathead minnows to the end of their first growing season (Chapter Two). This enhanced survival occurred despite the fact that more eggs were laid and hatched in the treatment pond sides than in reference sides. As a result of both greater production and greater survival, the average total numbers of fish that survived in treatment pond sides was 11, 113 compared to 1,922 in reference sides (Chapter Two). However although overall survival was enhanced it is not clear whether survival increased for all fish sizes and/or hatch-dates or for only certain sizes and or hatch-dates.

To address this question, in this chapter I used otolith microstructural analysis to examine survival of age-0 fathead minnows (*Pimephales promelas*) in experimental ponds subject to nutrient addition. I hypothesized that fish growing in ponds with additional nutrients (which should result in greater food availability) should have increased growth rates and therefore reduced size-selective mortality. If so, then in such ponds, length-frequency distributions of surviving fish at various points throughout the season (determined from back-calculation using otoliths) should differ less significantly from distributions from a random sample of fish collected at those same times, compared to reference pond side fish. I also hypothesized that juvenile mortality would vary with hatching date; the frequency distribution of hatching dates for end-of-season surviving fish would differ from observed hatching distributions. If

the age-0 fish that survive are unique with respect to body size or hatch date, this should assist in determining how mortality mechanisms act to regulate fathead minnow survival and year-class strength.

Prior to the use of otoliths in the back-calculation of fish lengths (Campana 1990) and hatch dates (Campana and Neilson 1985; Rice et al. 1987; Campana and Jones 1992), daily periodicity of otolith increments must be verified. Since daily periodicity has not been previously confirmed for fathead minnows, I conducted an enclosure experiment using known-age larval and juvenile fathead minnows to validate daily increment formation in this species.

Campana's (1990) biological intercept method for the back-calculation of fish length assumes that fish and otolith growth are proportional. Therefore, in addition to daily increment validation, I used fish from my pond study to determine if a linear relationship exists between otolith length and fish length.

3.2 METHODS

3.2.1 Natural History

Fathead minnows (*Pimephales promelas*) are short-lived (lifespan ≤ 3 years), small-bodied, cyprinids (Markus 1934; Scott and Crossman 1973). They occur throughout most of central North America, with their northern distributional limit at the Alberta-Northwest Territories' border (Scott and Crossman 1973). In Alberta, fathead minnows inhabit shallow lakes and ponds that often lack other fish, especially piscivores (Robinson and Tonn 1989). Fathead minnow diets include plant material (algae and macrophytes), detritus, zooplankton, and insects (Scott and Crossman 1973; Held and Peterka 1974; Tallman et al. 1984; Price et al. 1990). They can cannibalize their own young but are gape-limited predators, unable to consume young > 19 mm (Vandenbos 1996). In northern Alberta, spawning commences in late May, when water temperatures reach 15.6°C - 17.8°C (Scott and Crossman 1973) and continues until August. As a result of the protracted spawning season, fish hatch throughout much of the summer; therefore, newly hatched larvae experience different physical and biological environments depending on hatch date. Larvae hatch at an average length of 5 mm (Buynak and Mohr 1979), have yolk sacs, small mouths, and pigmented eyes (personal observation). Post-larval and juvenile fish remain preferentially in shallow, nearshore waters (personal observation).

3.2.2 Otolith Validation

I assessed daily otolith increment formation and the relation of otolith size to fish size in an enclosure experiment conducted at the Meanook Biological Research Station (MBRS; 54°37'N, 113°35'W). Eggs were collected in July from a nearby

natural pond. Spawning substrata, floating wooden boards covered with black tarpaulin, were anchored in positions along the shoreline. Within 2 d, eggs spawned on the boards were collected. All eggs were in the first stage of development as evidenced by the lack of pigmented eyes (Vandenbos 1996).

Eggs were transported to the laboratory where they were incubated in 5L buckets containing aerated water. The buckets were kept outside to expose eggs to natural light and dark cycles (16L:8D). During incubation, half of the water was replaced daily from each bucket. Eggs infected with fungus were removed daily with tweezers. Egg incubation lasted ca. 7 d and only larvae hatched within the same 24-h period were used in the experiment. On July-23, 1000 newly hatched (day-0) larvae were placed into each of three enclosures (2.5 m x 2.5 m x 0.6 m (deep)) located within a pond at MBRS. Fifteen day-0 fish from each enclosure were measured, euthanized with MS-222, and preserved in 95% ethanol. Fish were sampled at four dates (August 5 (day-13), August 15 (day-23), August 29 (day-37), and September 13 (day-52)) using an electrofishing unit and dipnet. The first 15 fish caught on each date from each enclosure were removed, measured (total and standard length) with an ocular micrometer to the nearest 0.1 cm and preserved (95% ethanol) individually in labeled vials for later otolith analysis.

3.2.2.1 Otolith Preparation

Lapillar otoliths, found in the utriculus of the pars superior in the membranous labyrinth of the inner ear, were removed from preserved fish and fixed on glass slides with thermoplastic cement. Prior to grinding and etching, length, width, and total area was measured for each otolith (Fig. 3-1). Length and width were measured

perpendicular to each other through the center of the otolith along the longest and shortest growth axes, respectively. All measurements were conducted with a light microscope (200x-1000x magnification) connected via a video camera to a personal-computer equipped with Optimas software.

Otoliths were hand ground with aluminum oxide (1500 grit) wet-dry sandpaper along the sagittal axis towards the midplane (Secor et al. 1992). To check for increments in the core and peripheral regions and prevent overgrinding, otoliths were frequently observed during grinding under the microscope. Once the side of the otolith was flat and increments were discernable, slides were reheated to melt the thermoplastic cement and the otoliths were flipped over with a probe. Otoliths were etched on each side immediately after grinding with 1 % hydrochloric acid for 1-2 minutes and rinsed with distilled water prior to flipping. The preparation process was repeated on the opposite otolith side.

3.2.2.2 Increment Counts

After grinding, the otolith core (hatch check) area and diameters (length and width), and the radius of the otolith from the focus to the periphery were measured and growth increments were counted using Optimas (Fig. 3-1). Increment interpretation is outlined in Campana (1992). Although several otoliths had a distinct hatch check easily distinguished from weaker pre- and post-hatch increments, most otoliths lacked obvious hatch-checks. To initiate counts on otoliths without clear hatch checks, or to confirm weaker hatch checks, mean day-0 (Table 3-1) dimensions (length and width) were used. All microzone counts were made along the longest axis of the otolith.

To facilitate increment counting, three zones were identified, classified according to the clarity of increments. Zone one (Z1), occurring immediately after the hatch check (6-12 increments), was the most difficult region to resolve since it was often over-ground in attempts to make other regions visible. Zone two (Z2) constituted the best-etched, most clearly resolved increments. Zone three (Z3), located on the periphery of the otolith, contained poorly resolved increments (5-10), also as a result of over-grinding. Focal depth of the microscope was adjusted for the different sections of the otolith to view all increments clearly.

Three increment counts were conducted per otolith and read "double blind" with both the known age or increment numbers from previous counts and size of the larvae unknown. Counts for each otolith were conducted in sessions separated by at least one week to avoid bias that could arise from familiarization of otoliths and their previous increment count. A qualitative code from 1 to 10 (with one being the best) was assigned to each otolith to indicate the reader's confidence in a count. Otoliths were discarded from analysis if either 1) successive counts were not within 5% of each other or 2) the qualitative rank assigned to a particular otolith was greater than 6. All three counts were averaged to provide a final increment number.

3.2.2.3 Comparisons of Corresponding Left and Right Otoliths

To determine if differences existed between corresponding right and left lapillar otoliths, I compared major otolith parameters (length, width and total otolith area) and number of increments using paired-*t*-tests. If otoliths were significantly different, judged by a $P \leq 0.05$, then the right and left lapillar otoliths could not be used interchangeably (Neilson 1992).

3.2.2.4 Daily Increment Validation

Regression analysis was used to determine the relationship between the number of increments and the known age of fish. Daily periodicity of otolith increments is accepted if the slope of the regression equation is significantly different from zero but not significantly different from one and the intercept is not significantly different from zero. Students' t-tests were used to test for significance of slope and intercept.

3.2.2.5 Otolith Length – Fish Length Relationship

Prior to using Campana's (1990) algorithm to back-calculate fish length in my pond study (see below), I verified the linearity between otolith growth and somatic growth. Otolith long radius and fish lengths were measured for age-0 fathead minnows that survived to the end of the summer (September 8) and were compared using regression analysis. The long otolith radius was used since this was the axis that was selected for back-calculation measurements.

3.2.3 Pond Study: Investigation of Selective Mortality

3.2.3.1 Study Site

Research was conducted in three experimental ponds (length: 25 m; width: 13 m; maximum depth: 1.5 m), located at MBRS. Each pond was divided in half, width-wise, to pair one treatment and reference pond side. Spawning substrata, consisting of eleven floating wooden boards (0.2 m x 1.5 m) covered by black tarpaulin and anchored with bricks, were added to each pond side.

3.2.3.2 Nutrient Addition

Beginning June 13 1996, one side each of three ponds was treated (nutrient addition), with the opposite side left as an unmanipulated reference. Nutrients, liquid

inorganic phosphoric acid (85% H_3PO_4) and dry ammonium nitrate (NH_4NO_3 (34%N)), were added four times (ca. monthly) throughout the summer. Quantities of nutrients were calculated to increase the treatment side total phosphorus (TP) 5 x compared with reference TP and to maintain the ratios of total nitrogen (TN) to TP at approximately 20:1. Phosphoric acid was added, on average, at concentrations of 80 µg/L-P and ammonium nitrate at 1200 µg/L-N. Application involved adding both nutrients separately to buckets containing 15L of pond water. These mixtures were then sprayed onto the pond surface and mixed vigorously with canoe paddles.

3.2.3.3 Stocked Fish

On June 15-16 1996, each pond side was stocked with identical size and life-history distributions and densities (1 fish / m^3 (148 fish / pond side)) of fathead minnows, within the range of distributions and densities found in nature (W.M. Tonn, personal communication). Relatively low densities were selected since, at these densities, high age-0 fish production occurs as a result of low rates of egg cannibalism (Vandenbos 1996). Therefore, if size-selective mortality is a mechanism influencing age-0 recruitment, evidence would be most pronounced at high age-0 densities, with corresponding high rates of intra-cohort competition (Vandenbos 1996).

3.2.3.4 Data Collection

3.2.3.4.1 Observed Egg Hatching Distributions

The number of eggs hatched in each pond side was estimated by mapping and quantifying egg batches and identifying developmental stages (see below) and states (alive, cannibalized, diseased) of eggs within all batches on nest boards on alternate days. Eggs were enumerated using a wire grid containing 20 1-cm^2 squares. After

placing the grid on top of an egg batch, the number of eggs in the core of the batch was determined by multiplying the number of squares covering eggs by the average number of eggs per square; the average number of eggs per square was previously determined from 20 counts of eggs, in different stages of development, within one square. Eggs located peripherally around the core were counted individually and added to the core number to get the total egg number for the batch.

The absence or presence of pigmentation in the eyes of the embryos differentiates egg developmental stages (Vandenbos 1996). The first stage is identified by the lack of eye pigmentation, in the second stage, eyes are pigmented black, and the final (third) stage is characterized by eyes that appeared gold due to retinal development. Eggs would typically hatch within 48 h of reaching the third stage.

Egg batches were classified as hatched if they disappeared on a sampling date after reaching the third (gold-eyed) stage. Batches disappearing suddenly (and usually entirely) in the first and second stage were considered cannibalized (Vandenbos 1996). Eggs infected with fungus were also recorded; these eggs would remain on the boards for a number of days but gradually disappeared.

3.2.3.4.2 Age-0 Fish

Age-0 fish were sampled at four dates (July 17, July 29, August 13, and August 29) from inshore (ca. <1m from shore) and offshore (ca. >3m from shore) habitats by electrofishing (See Chapter Two). On each date, a sample of approximately 30 fry per pond side was measured for total length, standard length and body depth to the nearest 0.1 mm. At the end of the season (September, 1996), ponds were drained to a maximum depth of 60 cm and all surviving fish were removed using a combination of

trapping and seining. All fish caught were killed (anaesthetic overdose) and preserved in 95% ethanol. A subsample of approximately 30 fish/pond side, used for otolith analysis, was measured for total length, standard length, and weight. Growth rates were calculated for individual fish by subtracting average standard length of fish at time of hatch (5.17 mm) from standard length at time of capture and dividing by the age of the fish (determined from otolith analysis). Growth rates were compared between treatment and reference pond sides using a paired *t*-test.

3.2.3.5 Size-Selective Mortality

Otoliths from a subsample of approximately 30 fish per pond side collected on September 8 were used to back-calculate lengths of each fish on the four earlier sampling dates (July 17, July 29, August 13, August 29). The back-calculated lengths were then compared with observed lengths of fish collected on these dates to determine if fish surviving to the end of the season had length distributions characteristic of the general population at earlier dates. The extent of any difference would indicate the intensity of size-selective mortality for each pond side.

Back-calculated lengths were determined using Campana's (1990) biological intercept procedure:

$L_a = L_c + (O_a-O_c)(L_c-L_0)/(O_c-O_0)$, where L_a = standard length at date *a*, L_c = standard length at the end of the season, O_a=otolith radius at date *a*, O_c = otolith radius at the end of season, L_0 = standard length at hatching (5.17 mm) and O_0 = otolith radius at hatching (24.74 μm; Table 3-1).

Otolith radius at date *a* (O_a) was measured along the posterior radius of the lapillus (consistently the best line for increment counting) from the center of the focal

area to the increment on the otolith that represented one of the four sampling dates (identified by subtraction from the date of capture (September 8,1996; Fig. 3-2). Length distributions of age-0 fish on the four sampling dates were obtained from the biweekly electrofishing in each pond side. Finally, back-calculated length-frequency distributions were compared to observed distributions with the Kolmogorov-Smirnov two-sample test (Sokal and Rolf 1981). Significant differences would indicate that size-selection had occurred.

3.2.3.6 Hatch-Date-Selective Mortality

Otolith analysis was also used to determine the hatch-date distributions of age-0 fish that survived to the end of the season. Increments were counted for each of 30 fish per pond side and hatch date was calculated as the date of capture (September 8, 1996) minus the total number of increments (age). The back-calculated hatch-date distributions were then compared to the observed hatching frequency distributions, obtained from egg sampling throughout the season, using the Kolmogorov-Smirnov two-sample test to determine if hatch dates of survivors differed from the distribution of the entire cohort.

3.3 RESULTS

Of the three pairs of otoliths in the inner ear of the fish, the lapillus was the most suitable for aging and growth studies of age-0 fathead minnows. Similar to other cyprinid otoliths, the lapillar otolith in fathead minnows was the largest and least brittle and also developed in the embryo prior to hatch (Victor and Brothers 1982; Campana and Neilson 1985). At hatch both the lapillar and sagittal otoliths were present. The lapillar otoliths were spherical, with a diameter of 36.3 - 60.8 μm ($\bar{x}$=48.6 μm; Table 3-1) and contained several pre-hatch increments (Fig. 3-3). Within 13 days of hatch the asterisci otoliths were also present.

Otolith preparation was required for all otoliths larger than 191 μm. Preparation dramatically improved resolution of increments (Fig. 3-4). One growth increment consisted of an incremental (wide and transluscent) zone and a discontinuous (narrow and opaque) zone. Subdaily growth increments consisted of faint discontinuous zones. When increments were difficult to resolve within short segments (<50 μm) of the otolith, the number of increments for these regions was interpolated. The poorly resolved section was measured and multiplied by the average number of rings per length in 20 μm segments immediately before and after the poorly resolved section. Interpolation involved ca. 25% of the increments observed.

3.3.1 Otolith Validation

3.3.1.1 Comparisons of Corresponding Left and Right Otoliths

Otoliths for known 0, 13, 43, and 44 day old fish were used to compare major otolith dimensions and increment counts between right and left lapillar otoliths. Paired *t*-test comparisons (*df*=70) were not significant between corresponding left and right

sides for length (t=-0.36, P=0.72), width (t=-0.53, P=0.60), and otolith area (t=-0.14, P=0.89). In addition, for 13, 43, and 44 day old fish, increment counts between corresponding left and right otoliths did not differ (t=0.04, df=43, P=0.97).

3.3.1.2 Daily Increment Validation

Although aging was attempted for all fish from the validation experiment, less than one-quarter of fish older than 13 d had readable otoliths. Otoliths from these fish required preparation; however, the otoliths were extremely brittle and preparation was generally unsuccessful. Furthermore, increments were extremely weak (Fig. 3-5), resulting in low confidence in counts. Fragile otoliths and poor banding likely resulted from the low productivity found in these enclosures. Therefore, for the validation of daily increments I used only day-13 otoliths and additionally used known-age (day-43 and day-44) fish from a second enclosure experiment (Chapter four) since these fish had high food rations (supplemented with *Artemia* nauplii).

When the number of increments counted on lapillar otoliths was regressed against the known age (in days) (Fig. 3-6), the following relationship was obtained:

Increment Count=0.91 (Age) – 0.06, n=45, r^2=0.99, P<0.001

The slope was different from both zero and one, but the intercept was not different from zero. The 95% confidence interval for estimating the age of an individual fish from its increment count using the inverse prediction method was ± 2.7 days (Sokal and Rolf 1981).

3.3.1.3 Otolith Length – Fish Length Relationship

Standard length was regressed on otolith length for fish that survived to the end of the summer (September 8); fish were pooled from samples obtained from each pond

(both treatment and control sides). The following relationship between log(standard length) and log(otolith radius) was linear (Fig. 3-7):

log (Standard Length (mm)) = 1.32 log ((Otolith Length (μm))) – 2.18,

n=166, r^2=0.95, P<0.001

The 95% confidence interval for estimating standard length of an individual fish from its otolith radius was ± 0.068 (mm).

3.3.2 Pond Study: Investigation of Selective Mortality

On average, 72% of age-0 fish sampled during the pond experiment were aged successfully using either the left or right lapillus. Otoliths from pond two were more difficult to age due to weaker increment formation compared with other ponds. Since the validation experiment yielded a slope for the known age-otolith increment relationship slightly, although significantly, below one, an inverse regression method (Sokal and Rohlf 1981) was used to calculate ages of individual fish surviving to the end of the season. Average growth rates of age-0 fish were not significantly different between treatment and reference pond sides (Fig. 3-8).

3.3.2.1 Size-Selective Mortality

To determine the amount of shrinkage in juvenile fathead minnows preserved in 95% ethanol, c.a. 30 fish each were collected from the validation experiment on day-0, 13, 23, 30, and 44 and measured (standard length (mm)) while alive and then after 5 months of preservation. Fresh standard lengths of these fish were regressed on preserved standard length (preserved for 5 months) and the following relationship was acquired and used to correct for shrinkage to back-calculate size at age:

Fresh Standard Length (mm) = 1.08 (Preserved Standard Length (mm))-0.203

n=130, r^2=0.98, P<0.001

Correcting for shrinkage in preserved fish was used in the back-calculation of length of surviving fish, since back-calculation requires accurate fish lengths at the time of capture.

To determine if mortality in age-0 fish was selective with respect to body size, back-calculated lengths of survivors were compared with observed lengths of fish collected on four earlier dates. Distributions were significantly different for 5 of 12 comparisons from treatment pond sides (3 of 4 for pond 2) and for 5 of 11 comparisons from reference sides (2 of 3 from pond 2) (Table 3-2; Fig. 3-9 to Fig. 3-14).

3.3.2.2 Hatch-Date-Selective Mortality

Hatch-date distributions of fish surviving to the end of the first growing season (September 1996), calculated from otoliths, were compared with observed hatch-date distributions obtained from egg data, separately for each pond side. In the treatment pond sides, hatch-date distributions for surviving fish were not significantly different from observed egg hatching distribution in the field (K-S two-sample test, Pond One: Z=1.28, P=0.08; Pond Two: Z=0.94, P=0.34; Pond Three: Z=0.97, P=0.30)(Fig. 3-15). In contrast, back-calculated hatch-date distributions from surviving fish were significantly different from the observed hatching period in reference sides of ponds 1 and 3, with hatch-dates of survivors concentrated on dates after peak spawning (ca. July 6) (Pond One: Z=3.73, P<0.001: Pond Three: Z=2.44, P<0.001)(Fig. 3-16). In pond two reference pond side, where observed hatching was delayed until mid/late July, hatch dates of surviving fish were also significantly different from observed hatching

distributions; with hatch-dates of surviving fish concentrated prior to peak spawning in this pond side (ca. July 19) (Pond Two: Z=1.84, P=0.002).

3.4 DISCUSSION

3.4.1 Otolith Validation

In my study, no differences were observed in major dimensions and counts between corresponding left and right otoliths; therefore future studies on age-0 fathead minnows can use left or right lapillar otoliths interchangeably. Since differences between left and right otoliths have been reported for other species (see Neilson 1992), quantifying a lack of differences is necessary prior to using corresponding otoliths interchangeably.

It is not uncommon for observed increment counts to be slightly less than one increment per day when using light microscopy, as was found in age-0 fathead minnows in my validation study (e.g., Rice et al. 1985, 1993; Jones and Brothers 1987; Ruzicka and Radtke 1995). Under-estimation of fish age from otolith increments counts can be attributed to narrow increments found on certain poorly resolved otolith sections (Campana et al. 1987; Neilson 1992). When growth rates are relatively slow, due to lower temperatures or suboptimal feeding conditions, individual increments fall below the resolution limit of light microscopy (<1μm) (Jones and Brothers 1987; Savoy and Crecco 1987; Campana 1989). Observations of less than one increment per day can likely be attributed to methodological limitations (use of light-microscopy) rather than deviations from the expected daily rate of increment formation.

In my study, increments for fish with slow growth rates (<0.21 mm/day) were narrow and unclear in all zones (Z1-Z3) on the otolith and therefore increment counts could not be conducted with any degree of accuracy or precision. Although fish with faster growth rates (>0.21 mm/day) overall produced clear, well-defined increments,

those near the core (Z1) and in the periphery (Z3) of the otolith were weak, possibly accounting for the less than one-increment per day deposition rate observed in my study.

My study suggests that individual age-0 fathead minnows can still be estimated as long as fish were not growing at very low rates (<0.21 mm/day). The ability to age young fathead minnows permitted the determination of hatch dates, average growth rates, and back-calculation of lengths for fish in my pond study using the inverse prediction method (Sokal and Rohlf 1981).

In addition to providing evidence for daily increment formation, the proportionality between otolith length and somatic length is the second assumption of Campana's (1990) biological intercept method for back-calculation of fish lengths. I demonstrated here that in age-0 fathead minnow otoliths the relationship between total fish length and otolith length was linear. This justified the use of Campana's (1990) biological intercept method for back-calculation in the pond study.

The biological intercept method uses an intercept value that is determined biologically, based on the average fish and otolith length at hatch when both fish and otolith growth become proportional (Campana 1990). In contrast, Fraser-Lee's method of back-calculation uses an intercept value that is estimated statistically, based on the regression of fish-otolith length. Both Campana and Fraser-Lee's method of back-calculation assume proportionality between fish and otolith length. However, Campana's (1990) approach has one important advantage over Fraser-Lee's method of back-calculation (Campana 1990), in that it does not require the fish-otolith length relationship to be consistent for fish growing at different rates (Campana and Jones

1992). Since there is increasing evidence showing that slow-growing fish have larger and heavier otoliths compared with fast-growing fish of the same size (Secor and Dean 1989; Reznick et al. 1989; Campana 1990), the Fraser-Lee method would lead to underestimation of fish lengths at earlier dates.

3.4.2 Pond Study: Investigation of Selective Mortality

Otolith microstructural analysis generally provided an effective tool to assess the influence of size- and hatch-date-dependent mechanisms on the differences in age-0 fathead minnow survival between pond sides.

3.4.2.1 Size-Selective Mortality

In a study by Gleason and Bengtson (1996), mortality in a cohort of age-0 inland silversides (*Menidia beryllina*) was selective for larger individuals. In contrast, Post and Prankevicius (1987) found that mortality in a cohort of age-0 yellow perch (*Perca flavescens*) was selective for smaller, slower-growing individuals. Size-selective mortality in both studies was attributed to the selection of specific sizes of age-0 fish by predators. In a population of fathead minnows, where cannibalistic adults are likely the most important predators, size-selective mortality might focus on smaller, slower growing age-0 fish. Adult fathead minnows are gape-limited predators and cannot eat fish > 19mm TL (Vandenbos 1996). Therefore, slower growing fish will spend more time in sizes vulnerable to predation by adults. However, in the pond study size-selective mortality was not regularly observed. When it was observed it is more likely that these differences can be attributed to the small sample sizes used to construct length-frequency distributions for both observed fish lengths and back-calculated fish lengths. When sample sizes are small the sample mean is less likely to be close to the

population mean (Gravetter and Wallnau 1992). As a result, differences between two samples (observed and back-calculated lengths) are less likely to reflect actual differences. My study, however, does provide evidence that back-calculation of size-at-date, based on Campana's (1990) biological intercept method, can provide reasonable estimates of body size at earlier dates as evidenced by the proximity of back-calculated lengths with observed fish lengths. Future studies attempting this technique should use larger sample sizes and also sample age-0 fish starting from the time hatching commences to the end of the study period. This will be important since periods of intense mortality might be missed by not sampling throughout the entire range of dates when fish were alive.

3.4.2.2 Hatch-Date Selective Mortality

The instability of hatch-date distributions calculated from otoliths is the most important problem associated with their use (Campana and Jones 1992). In the calculation of hatch-date distributions from a sample of fish, younger fish (those hatched later in the season) should have experienced less cumulative mortality than older fish (those hatched earlier in the season). As a result, younger fish may be overrepresented in calculated hatch-date distributions compared to older fish (Campana and Jones 1992). However, since mortality of age-0 fish generally decreases with age, the time when all fish in a population reach an age when mortality rates are relatively low, calculated hatch-date distributions should reflect actual distributions rather than differences in cumulative mortality between early and late-hatching fish (Campana and Jones 1992).

Generally, since mortality in age-0 fish is most intense during the first few weeks of life (Crecco and Savoy 1985; Rice et al. 1987), fish used for hatch-date analysis sampled after this period should have corresponding low mortality rates. In my study, since fish sampled at the end of the season (September 8) were a minimum of 31 days old, mortality of even the youngest fish should have been low by this time. As a result, hatch date distributions calculated from the otoliths of surviving fish should have been stable (Campana and Jones 1992). Therefore, differences between the observed egg hatching distributions compared with hatching distributions calculated from a subsample of surviving fish otoliths should reflect actual hatch-date selection rather than differences in cumulative mortality between youngest and oldest larvae.

In my study, difference between observed and calculated hatching distributions occurred only in the reference pond sides. Specifically, mortality was most intense prior to and during peak spawning (ca. July 6) for pond one and three reference pond sides and after peak spawning (ca. July 18) for pond two. However, in pond two the differences between observed hatch-date distributions and calculated hatch-date distributions from otoliths of surviving fish may also result from the small sample sizes of calculated hatch-date distributions rather than actual differences.

Other studies have also shown that when recruitment is poor, survival may be selective for hatch date (Crecco and Savoy 1985; Rice et al. 1987; McGovern and Olney 1996; Bulak et al. 1997). Lower water temperatures, decreased food availability, or increased predation may cause greater mortality at different times in the growing season (Campana and Jones 1992). McGovern and Olney (1996) found that survival in an average recruitment year was selective with respect to hatch date; most survivors

hatched in the second half of the spawning season when egg production was low. In contrast, in a year of high recruitment, survival was not selective. They attributed this difference to temporal differences in temperature regimes, food availability, and predation intensity during and following the spawning season between years, which resulted in fish hatching at certain times during the average recruitment year to be especially vulnerable to predation.

Since densities of potential predators (adult fathead minnows) and temperatures were similar among pond sides, the main factor that varied between treatment and reference sides was food availability. Phytoplankton and other plant material may be an important food source for fathead minnows as indicated by their long intestines (Scott and Crossman 1973). Several studies found that algae are a major food item of all life-history stages of the fathead minnow, including smaller juveniles (see Held and Peterka 1974). Indeed, Shaw et al. (1995) found that Bacillariophyta, filamentous algae and macrophytes comprised the greatest proportion of the diet of juvenile fathead minnows. Although other studies show that zooplankton and insect larvae can be the main food source for fathead minnows (Held and Peterka 1974; Price et al. 1990), this species has been referred to as the "temperate generalist" (see Tallman et al. 1984). Fathead minnows appear to be flexible in their choice of temporarily available food items. Consequently, seasonal changes in chlorophyll *a* (Chl*a*), an indicator of phytoplankton biomass, between and within pond sides likely influenced the food directly available to newly hatched fathead minnows.

Although Chl*a* concentrations in both treatment and reference pond sides increased in the second half of the season, differences in Chl*a* concentrations between

pond sides were more dramatic in the second half of the growing season. Following the initiation of nutrient additions on June 13 until mid-July, Chl*a* concentrations were three fold greater in the treatment ($\bar{x}$=6.8 µg/L) versus reference ($\bar{x}$=2.1 µg/L) pond sides. However, in the second half of the season, Chl*a* concentrations were seven fold greater in the treatment ($\bar{x}$=53.7 µg/L) versus reference ($\bar{x}$=7.5 µg/L) pond sides (Chapter Two).

Greater mortality observed in the reference ponds (1 and 3) during the first half of the season may thus be attributed to lower food availability compared with both the treatment sides and the second half of the season in the reference sides. An enclosure study showed that lower food availability, in the absence of predators, resulted in significantly slower growth rates in age-0 fathead minnows (Chapter Four). As a result, the lowest growth rates in age-0 fish for all pond sides should have occurred in the first half of the season in the reference pond sides. Since adult fathead minnows are gape-limited predators (Vandenbos 1996), slower growing age-0 fathead minnows should exceed sizes vulnerable to predation more slowly and therefore experience more mortality than faster growing fish. Smaller fish also have a reduced ability to detect and avoid predators and to capture food (Miller et al. 1988) due to associated slower swim speeds (Fernald 11988) and shorter reactive distances (Yates 1983). Therefore, the slower a fish grows the more time they spend at smaller body sizes that are more susceptible to several sources of mortality.

The intensity of cannibalism on age-0 fish might also have been influenced by the availability of alternative food for adult fathead minnows; a decrease in cannibalism can occur when alternative food availability increases (Margulies 1990; Gotceitas and

Brown 1993). Since food availability (as suggested by Chl*a*) was lowest in the first half of the season in the reference pond sides, mortality at this time might also have been influenced by an increase in cannibalism.

3.4.3 Conclusions

Results from my pond study show that otoliths can provide insights into the mechanisms influencing survival in age-0 fathead minnows. In two of three ponds, nutrient enhancement increased survival of age-0 fish in the first half of the hatching period, enabling more age-0 fathead minnows to survive to the end of the first growing season. This suggests that intra-seasonal variability of growth rates in age-0 fish, influenced by changes in food availability, can moderate the intensity of mortality due to starvation or predation and hence regulate year-class strength in fathead minnow populations. This result, when combined with the observed changes in pond primary productivity that resulted from the nutrient additions used in this study, can help forecast the response of northern fish populations to increasing anthropogenic stresses.

Increased nutrient loads for aquatic ecosystems are anticipated, particularly for northern latitudes, due to predicted climate and land-use changes. In response to increased anthropogenic activities, chiefly the burning of fossil fuels, a doubling of atmospheric concentrations of CO_2 and a resultant increase in global temperatures and precipitation is predicted (Hansen et al. 1984). Associated with the predicted increase in precipitation due to climate change, particularly in northern latitudes (Hansen 1991), runoff and nutrient loads to aquatic ecosystems should also increase (Waggoner 1990; Carpenter et al. 1992). Increases in logging and agriculture should also increase

nutrient loads to lakes (Sharpley and Smith 1993; Schlesinger 1997) in northern Alberta.

If productivity increases throughout a hatching period, then mortality should be more evenly distributed throughout the season with no substantial differences in the intensity of mortality for either early- or late- hatching fish. Further, if more fish survive, particularly from the first half of the season, they will have a longer period of time to grow and should be larger at the end of the first growing season. Larger fish are less susceptible to overwinter mortality, due to higher energy reserves and lower standard metabolic rates (Brett and Groves 1979; Shuter and Post 1990), so it follows that more fish should survive their first winter. Therefore, my experiment suggests that an increase in productivity should increase year-class strength for fathead minnow populations in northern Alberta.

3.4.4 Future Studies

3.4.4.1 Otolith Validation

My study suggesting that fathead minnow otoliths produce increments on a daily basis represents a preliminary analysis of the relationship between otolith increment deposition and age of age-0 fathead minnows. The validation study that I attempted for a range of ages (0, 13, 23, 37, 50) should be repeated under conditions where food availability does not limit the resolution of increments on otoliths of age-0 fish; only when fish (>13 days) were reared in enclosures with supplemental food (*Artemia*) did otoliths produce increments visible under the light microscope. Outdoor enclosures are the optimal environment for conducting validation studies with natural temperature and photoperiod conditions. Laboratory studies on otolith validation are less desirable since

they generally lack natural daily fluctuations in temperature, feeding, etc. that can effect the frequency of otolith increment deposition. As a consequence, otolith increments produced by larvae reared in the laboratory are generally weaker than those in the wild (Geffen 1992).

Future otolith validation studies should incorporate a range of known-age fish greater than the maximum age observed in my study (44 days) (Geffen 1987, 1992). This is necessary to confirm that increment deposition and age remains proportional in older juveniles. To determine when increment formation commences (Geffen 1987, 1992; Neilson 1992), increments prior to hatching and in days immediately after hatching should be observed. Another technique that might be useful to determine when to initiate increment counts is the marking of newly hatched fish otoliths with florescent compounds, such as tetracycline or alizarin complexone (Geffen 1992). These compounds are incorporated into the otolith within one day of exposure and are visible under ultraviolet light. Therefore, they can assist in the determination of the number of increments formed before hatch and help to confirm hatch check locations.

In addition, the validation of increment periodicity should be conducted for a range of growth rates of age-0 fish by manipulating factors such as food and temperature. This will help determine how increment deposition might be modified in otoliths of fish in natural populations. Since increments for fish in the validation study underestimated known age, and fish growing slower than 0.21 mm/day did not produce any clear increments, it will be important to determine if these results can be attributed to the resolution limit of light microscopy. Scanning electron microscopy (SEM) should be used, in conjunction with light microscopy, to determine if this is true for

otoliths in slower growing age-0 fathead minnows (Campana et al 1987; Jones and Brothers 1987; Ruzicka and Radtke 1995). SEM could reveal increments below the limit of resolution of light microscopy that potentially contributed to under-aging my otoliths. Although SEM is costly and time consuming, under some conditions it may be necessary to improve the aging accuracy.

3.4.4.2 Investigation of Mortality Mechanisms

Smaller-scale enclosure studies on newly hatched fathead minnows should be conducted to determine how variation in food availability influences growth rates and subsequent mortality due to predation and starvation (as proposed in Chapter Four). A broad food gradient, representing the range of food availability experienced in nature, should be used to determine, quantitatively, the range of mortality experienced by age-0 fish due to both starvation and predation. Future mechanistic studies should also determine how mortality due to predation by adult fathead minnows on age-0 fish varies in relation to abundance of alternative food.

The pond study should also be repeated with more replicates to provide a more rigorous examination of the effects of nutrient enrichment on mechanisms influencing mortality of age-0 fathead minnows. Further, the duration should be longer to determine if a new population equilibrium is established and if subsequent years are consistent with my initial observations. Also, to determine if slower growth in the first half of the season contributed to mortality of age-0 fish (as hypothesized in my study) growth of fish sampled throughout the season should be calculated using otolith microstructural analysis.

The combination of mechanistic enclosure studies, pond studies representing more natural ecosystems, and studies on natural populations can help to quantitatively describe the factors and mechanisms underlying recruitment variation in fishes. The results of the current study and those of proposed studies will be particularly important if we are to understand and accurately predict the responses of natural fish population sizes to increasing climate and land-use changes.

Table 3-1. Measurements of the major parameters of day-0 fathead minnow otoliths (left lapillus) (μm) and fish lengths (mm).

Measurement (n=26)	Average	Standard Error	Maximum	Minimum
Otolith (μm):				
Length	48.6	1.29	60.8	36.3
Width	44.3	1.53	61.5	30.2
Centre (Focus) to Edge (O_0)	24.7	0.55	30.1	18.2
Total Otolith Area	1712	101	2916	859
Fish (mm):				
Total Fish Length	5.44	0.04	5.04	5.95
Standard Fish Length (L_0)	5.17	0.04	4.81	5.66

Table 3-2. *Z*-values and significance levels obtained from Kolmogorov-Smirnov two-sample tests for comparisons between observed length-frequency distributions and surviving fish (to September 8) calculated length-frequency distributions (from otoliths) for age-0 fathead minnows for four dates (July 17, July 29, August 13, and August 29).

Pond Number	Side	Date Jul-17	Jul-29	Aug-13	Aug-29
One	Treatment	1.1	1.1	1.2	1.4*
	Reference	1.1	1.0	1.6**	1.4*
Two	Treatment	1.8**	1.4*	1.44*	1.2
	Reference		1.5*	1.7**	1.3
Three	Treatment	1.0	1.5*	0.9	1.0
	Reference	1.2	1.0	1.7**	0.8

* = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$

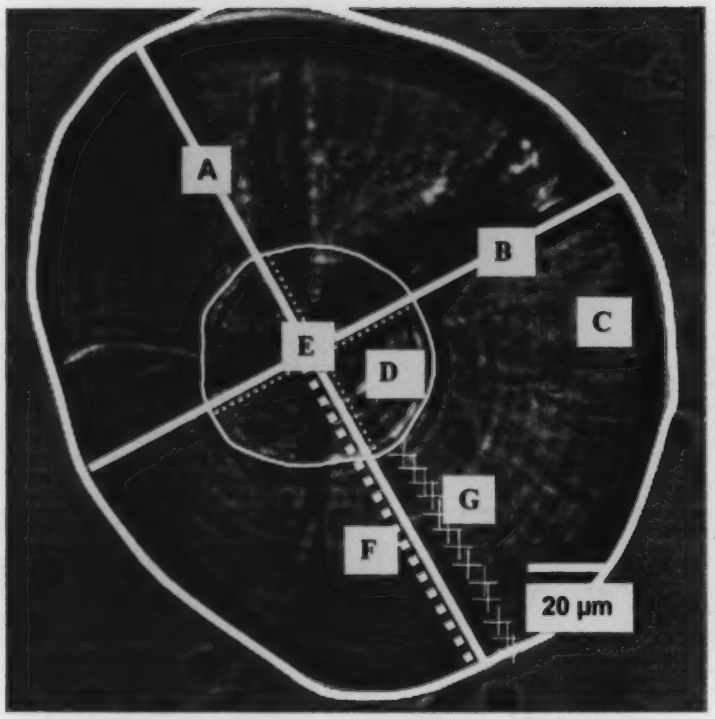

Fig. 3-1. Measurement of otolith **A.** length, **B.** width, **C.** total area (inside white line marking the total otolith perimeter), **D.** core area (inside white line marking the total core perimeter), **E.** core diameters, length and width (dotted-line), **F.** radius, from focus to periphery (dashed-line), **G.** daily growth increments (crosses), for an age-0 fathead minnow (ca. 13 days old), 400x magnification.

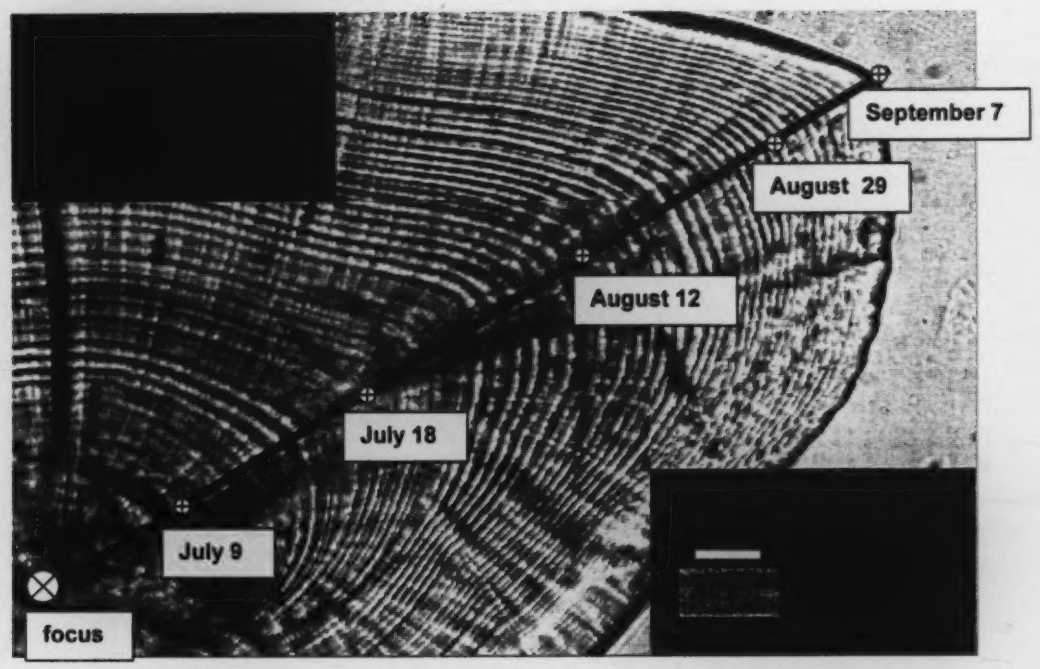

Fig. 3-2. Left lapillar otolith from fish number one, pond three (treatment side), 400x magnification. Back-calculated dates and focus (reference point) marked with circles.

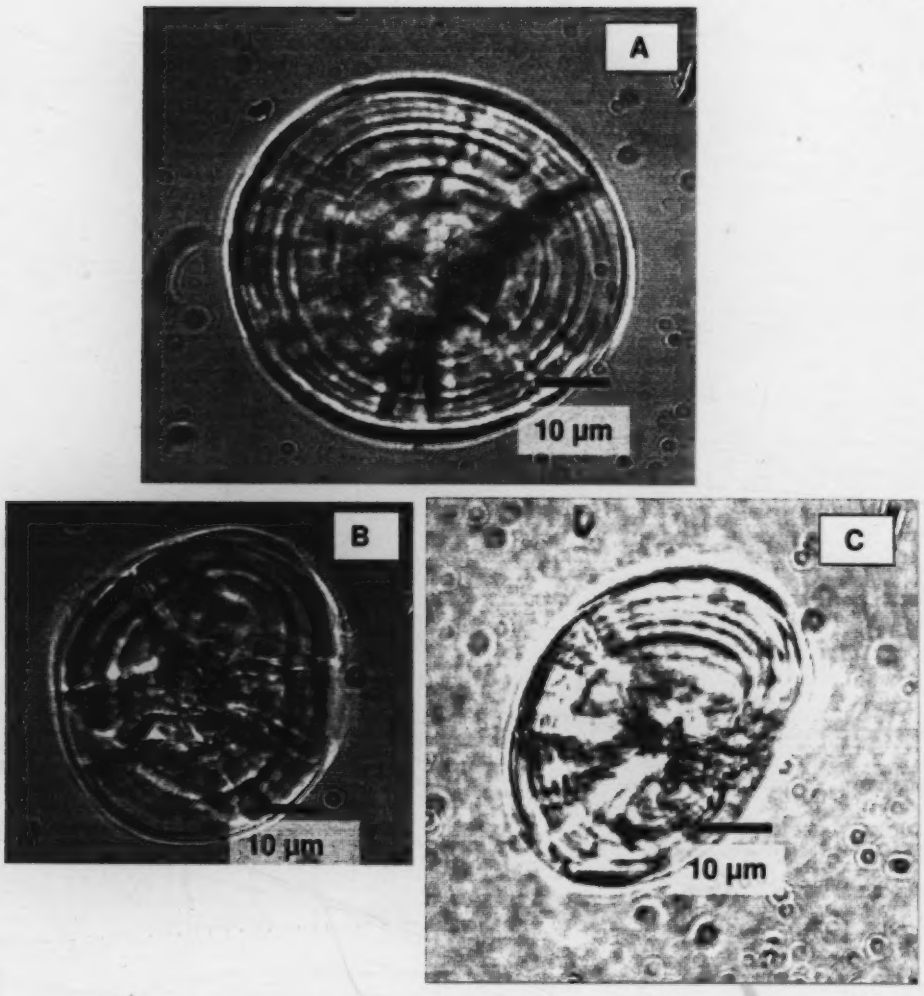

Fig. 3-3. Day-0 fathead minnow left lapillar otolith from three fish (A-C), 1000x magnification.

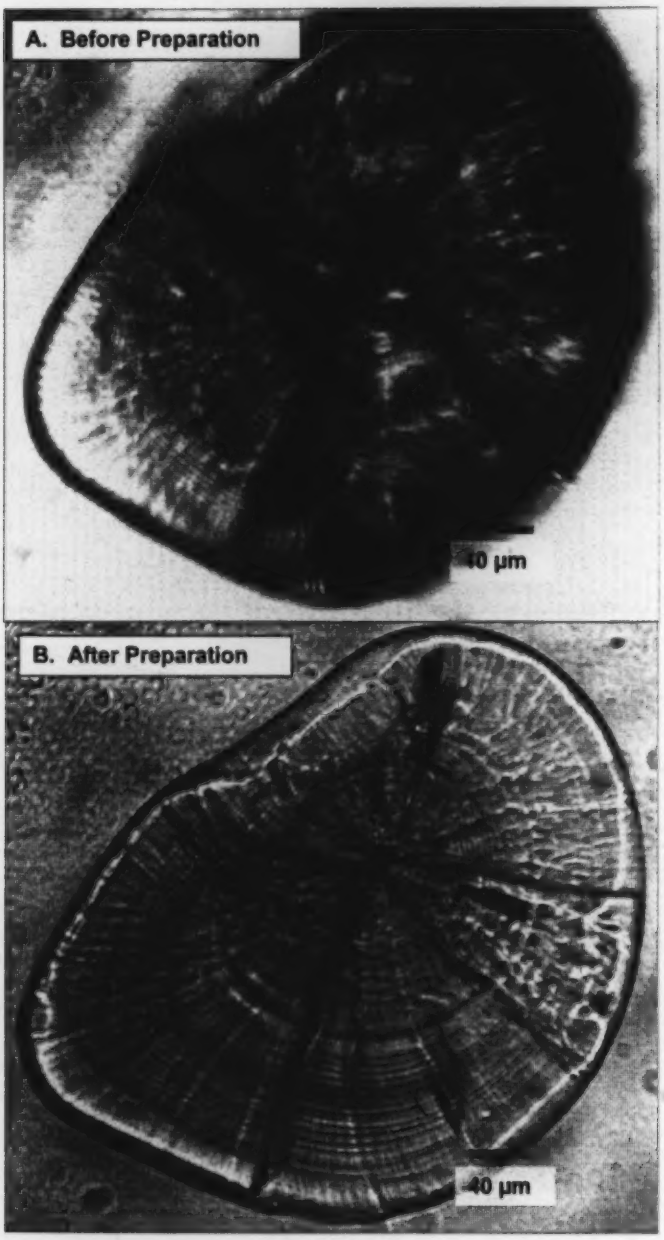

Fig. 3-4. Left lapillar otolith (A) before and (B) after preparation (grinding and etching), 200x magnification.

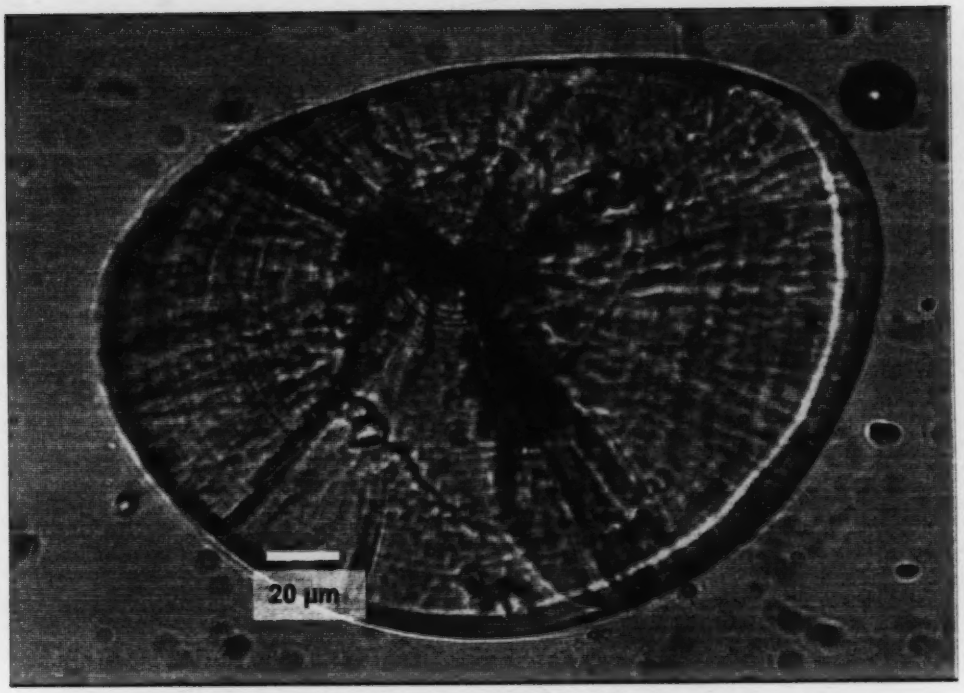

Fig. 3-5. Left lapillar otolith from validation experiment (day-23) under low food conditions, 400x magnification.

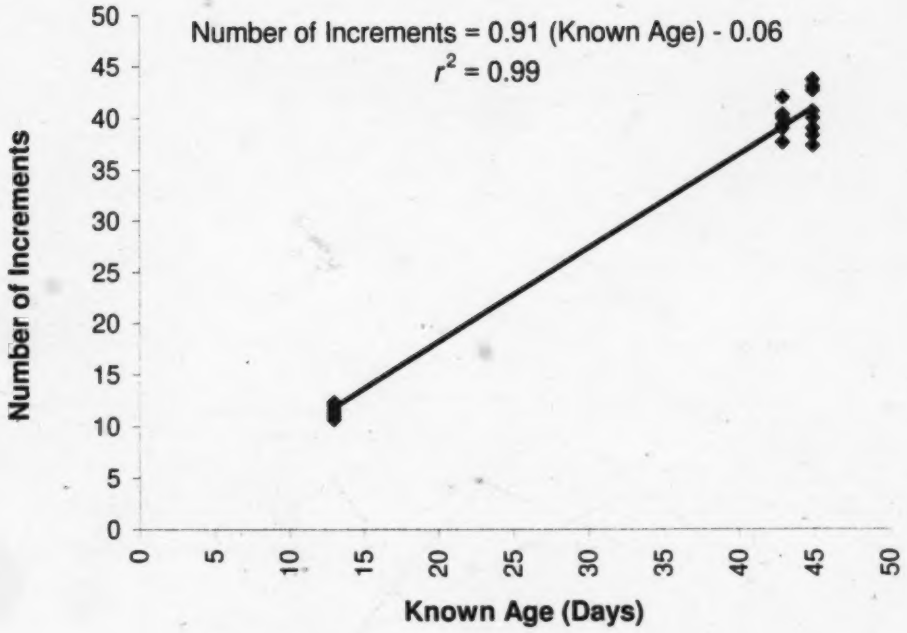

Fig. 3-6. Relationship between the number of otolith increments and the known age (days) for age-0 fathead minnows (n=45, r^2=0.99, P<0.001). The slope was different from both zero (t=80.0, P<0.001) and one (t=-7.78, P<0.001), but the intercept was not different from zero (t=-0.17, P=0.87).

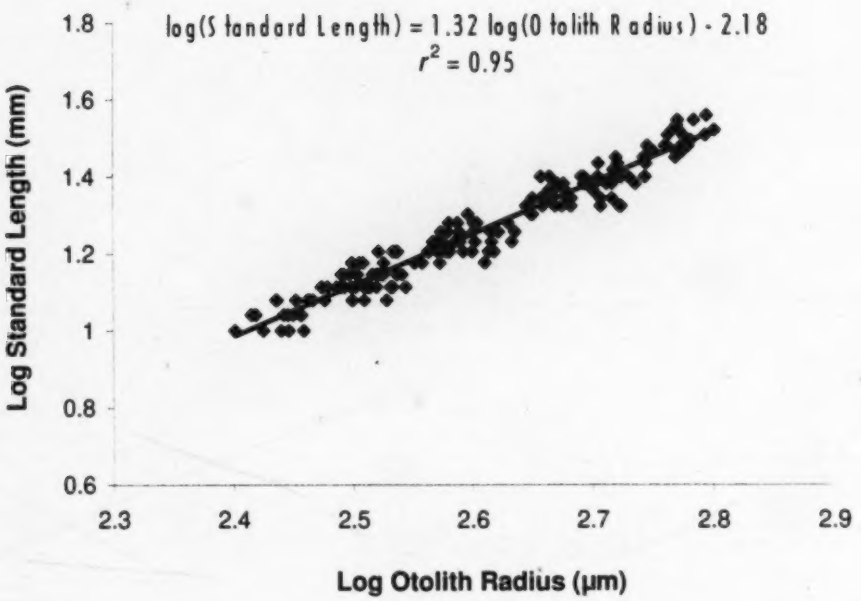

Fig. 3-7. Relationship between standard fish length (mm) and otolith radius (µm) (focus to periphery) for age-0 fathead minnows (n=166, r^2=0.95, P<0.001)

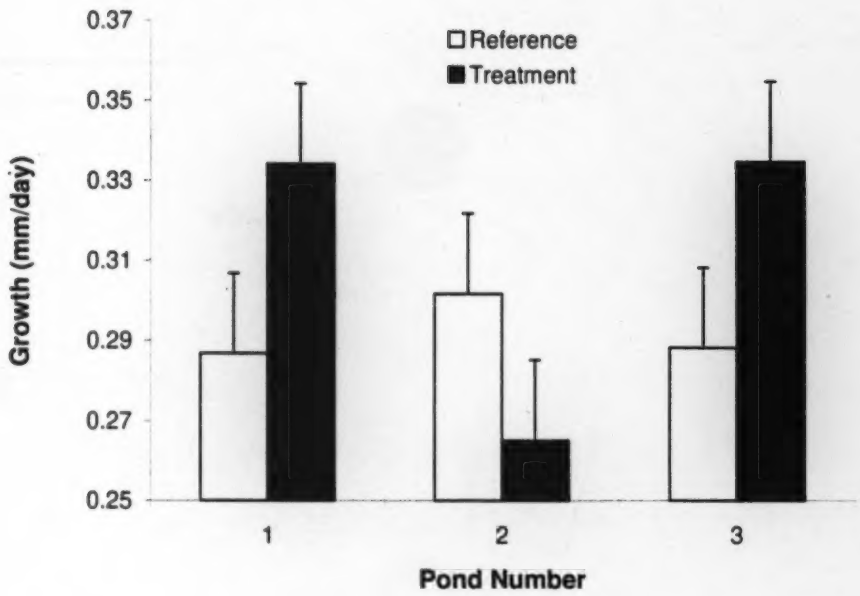

Fig. 3-8. Average growth rates (mm/day) (bars indicate one SE) (average standard length (SL) at hatch - SL at time of capture)/ fish age)) for surviving fish in all three ponds for treatment and reference pond sides (n=30/pond side). Growth rates were not significantly different between treatment and reference pond sides (paired t-test, t=0.56, df=2, P=0.63).

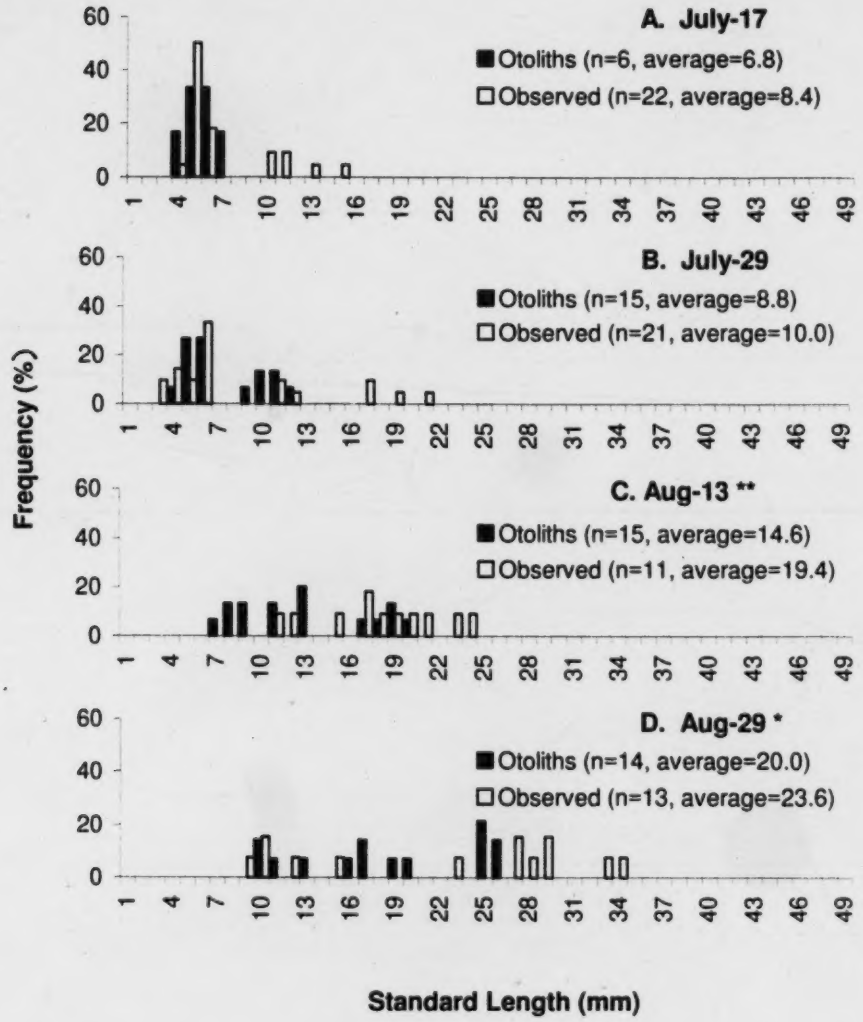

Fig. 3-9. Length-frequency distributions of age-0 fish from pond one (reference side) from four sampling dates (white bars), and distributions of back-calculated (from otoliths) lengths from the same dates for age-0 fish that survived to the end of the season (September 8, 1996)(black bars). Kolmogorov-Smirnov two-sample test (* = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$).

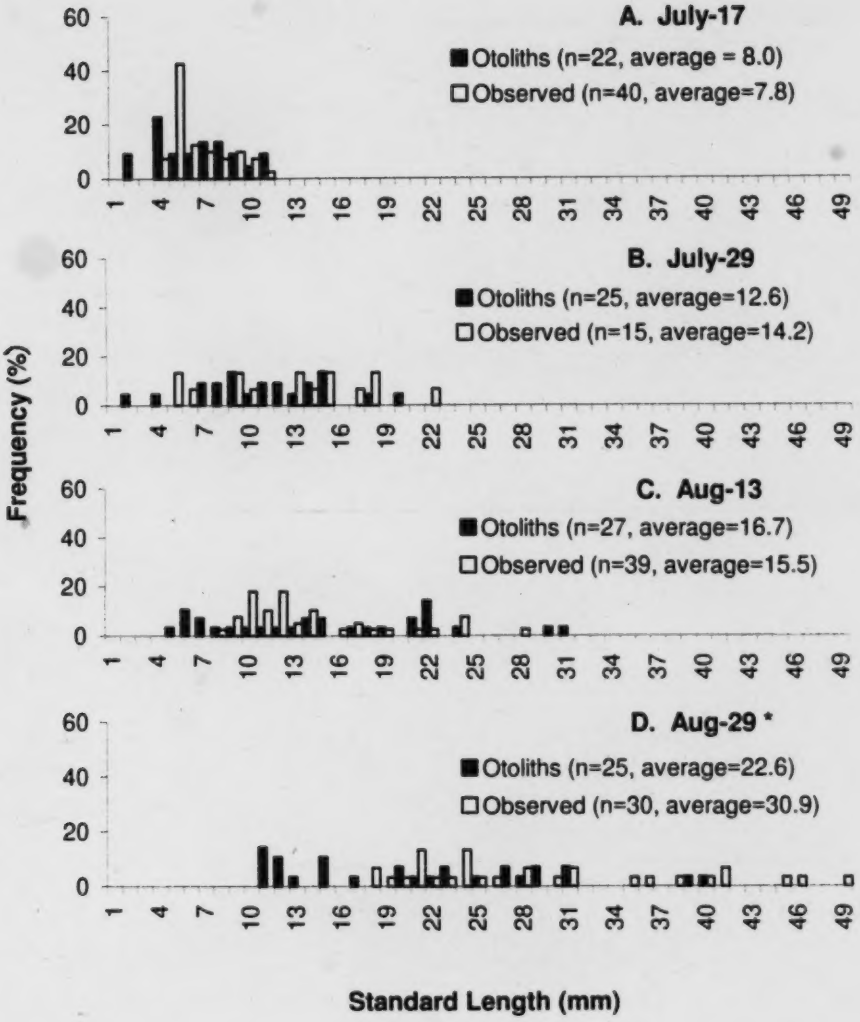

Fig. 3-10. Length-frequency distributions of age-0 fish from pond one (treatment side) from four sampling dates (white bars), and distributions of back-calculated (from otoliths) lengths from the same dates for age-0 fish that survived to the end of the season (September 8, 1996)(black bars). Kolmogorov-Smirnov two-sample test (* = $0.05 \geq P > 0.01$).

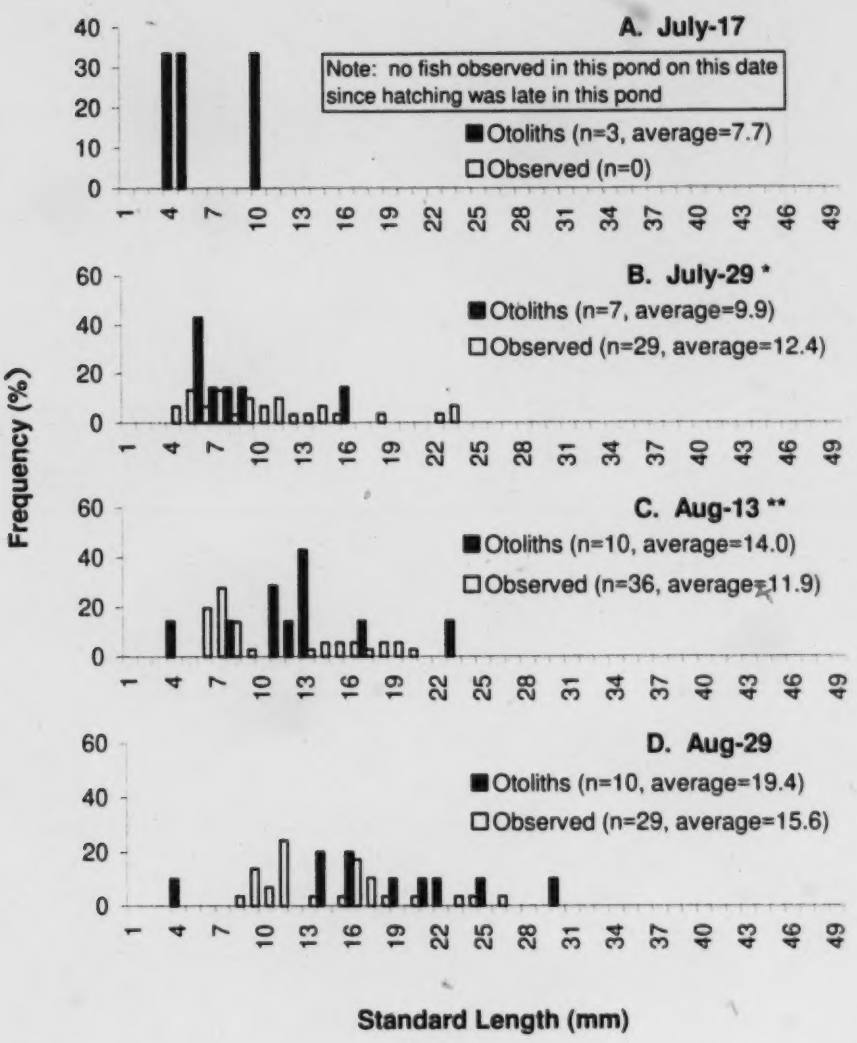

Fig. 3-11. Length-frequency distributions of age-0 fish from pond two (reference side) from four sampling dates (white bars), and distributions of back-calculated (from otoliths) lengths from the same dates for age-0 fish that survived to the end of the season (September 8, 1996)(black bars). Kolmogorov-Smirnov two-sample test (* = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$).

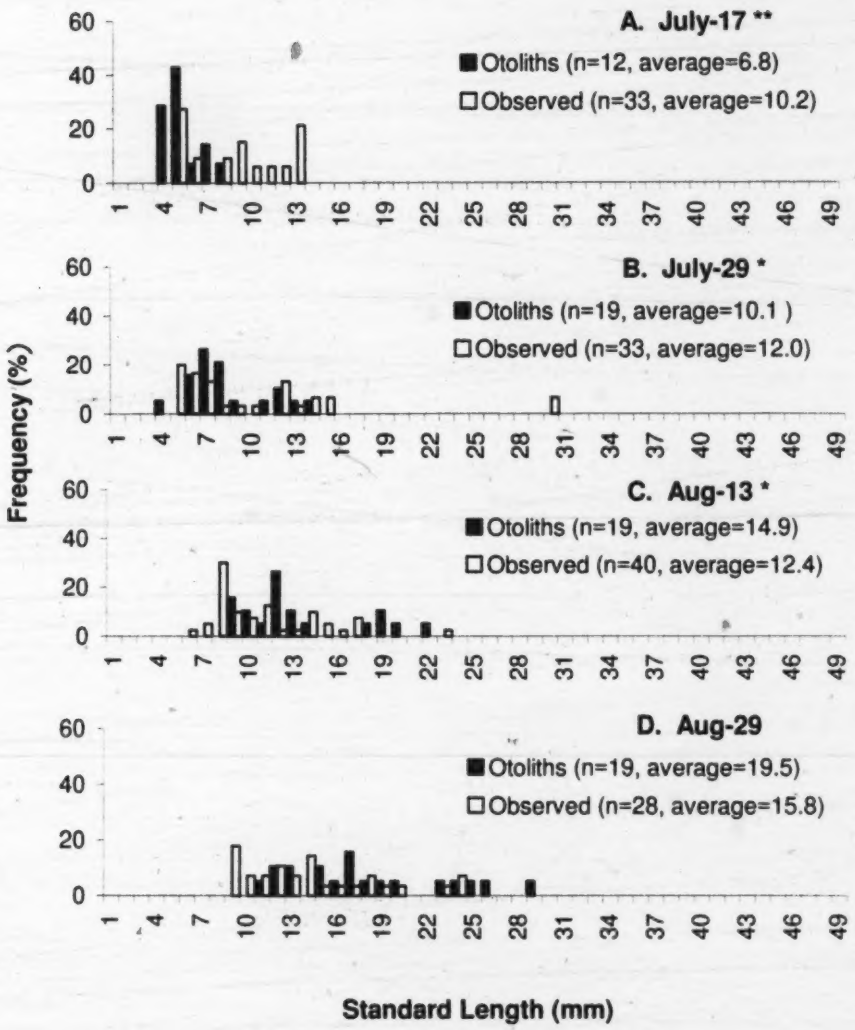

Fig. 3-12. Length-frequency distributions of age-0 fish from pond two (treatment side) from four sampling dates (white bars), and distributions of back-calculated (from otoliths) lengths from the same dates for age-0 fish that survived to the end of the season (September 8, 1996)(black bars). Kolmogorov-Smirnov two-sample test (* = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$).

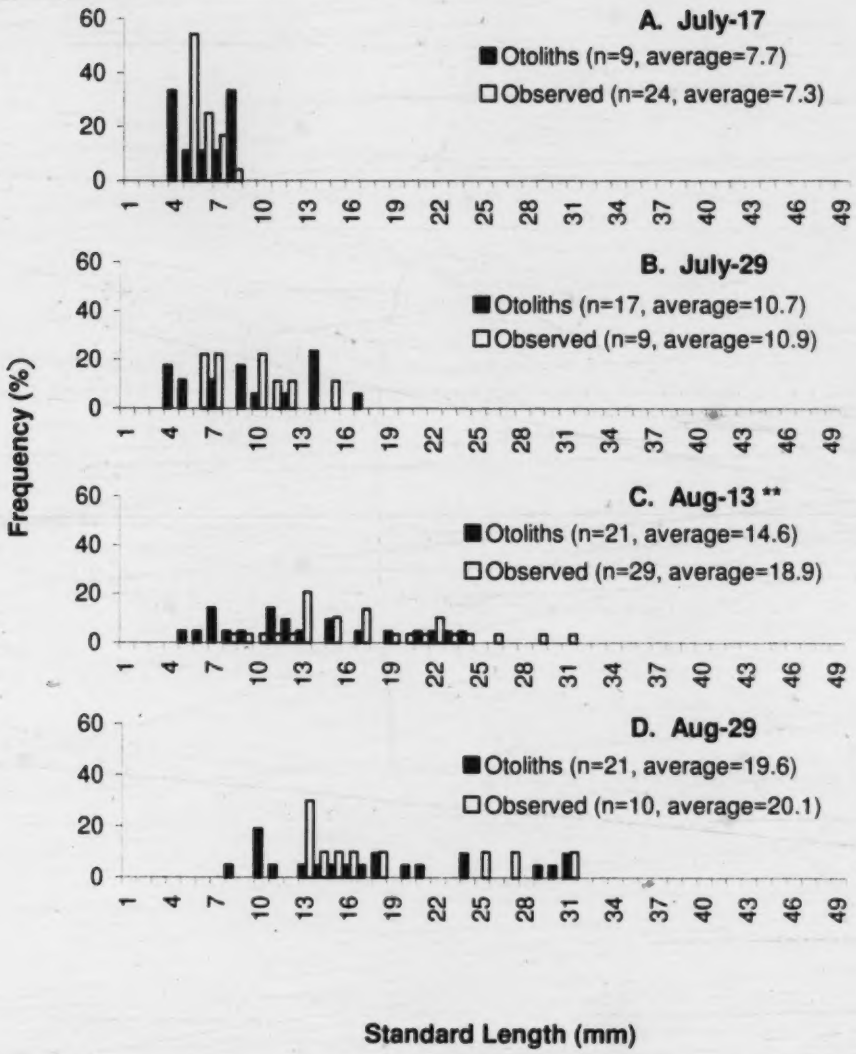

Fig. 3-13. Length-frequency distributions of age-0 fish from pond three (reference side) from four sampling dates (white bars), and distributions of back-calculated (from otoliths) lengths from the same dates for age-0 fish that survived to the end of the season (September 8, 1996)(black bars). Kolmogorov-Smirnov two-sample test (* = $0.05 \geq P > 0.01$).

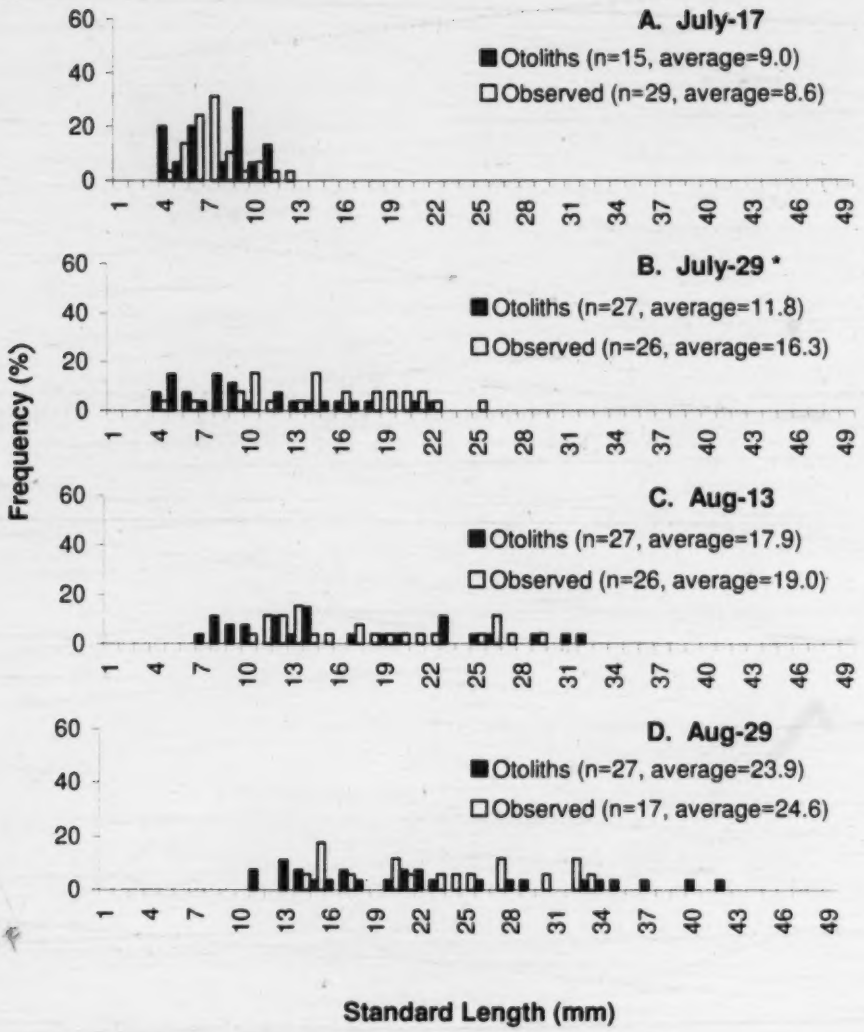

Fig. 3-14. Length-frequency distributions of age-0 fish from pond three (treatment side) from four sampling dates (white bars), and distributions of back-calculated (from otoliths) lengths from the same dates for age-0 fish that survived to the end of the season (September 8, 1996)(black bars). Kolmogorov-Smirnov two-sample test (* = $0.05 \geq P > 0.01$).

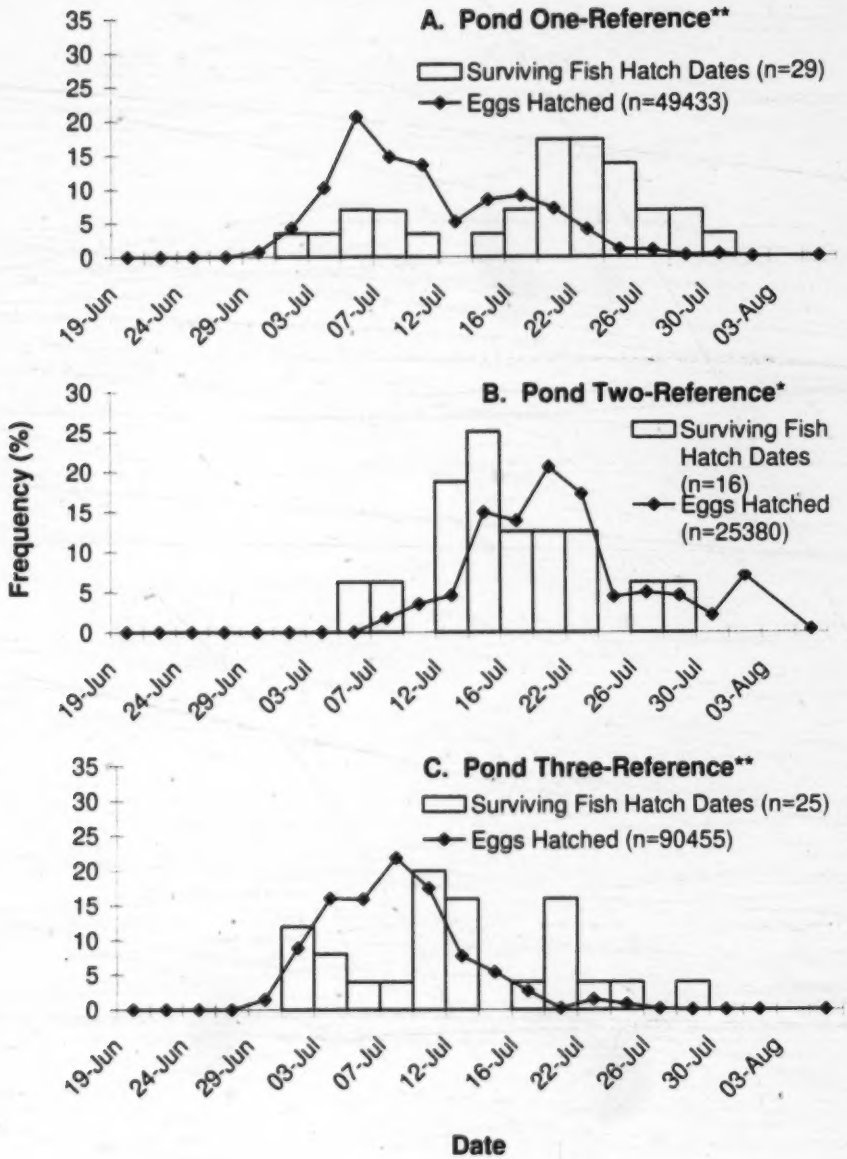

Fig. 3-15. Reference pond sides' observed egg hatching distributions compared with hatching distributions calculated from a subsample of otoliths from surviving fish otoliths. Kolmogorov-Smirnov two-sample test (* = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$).

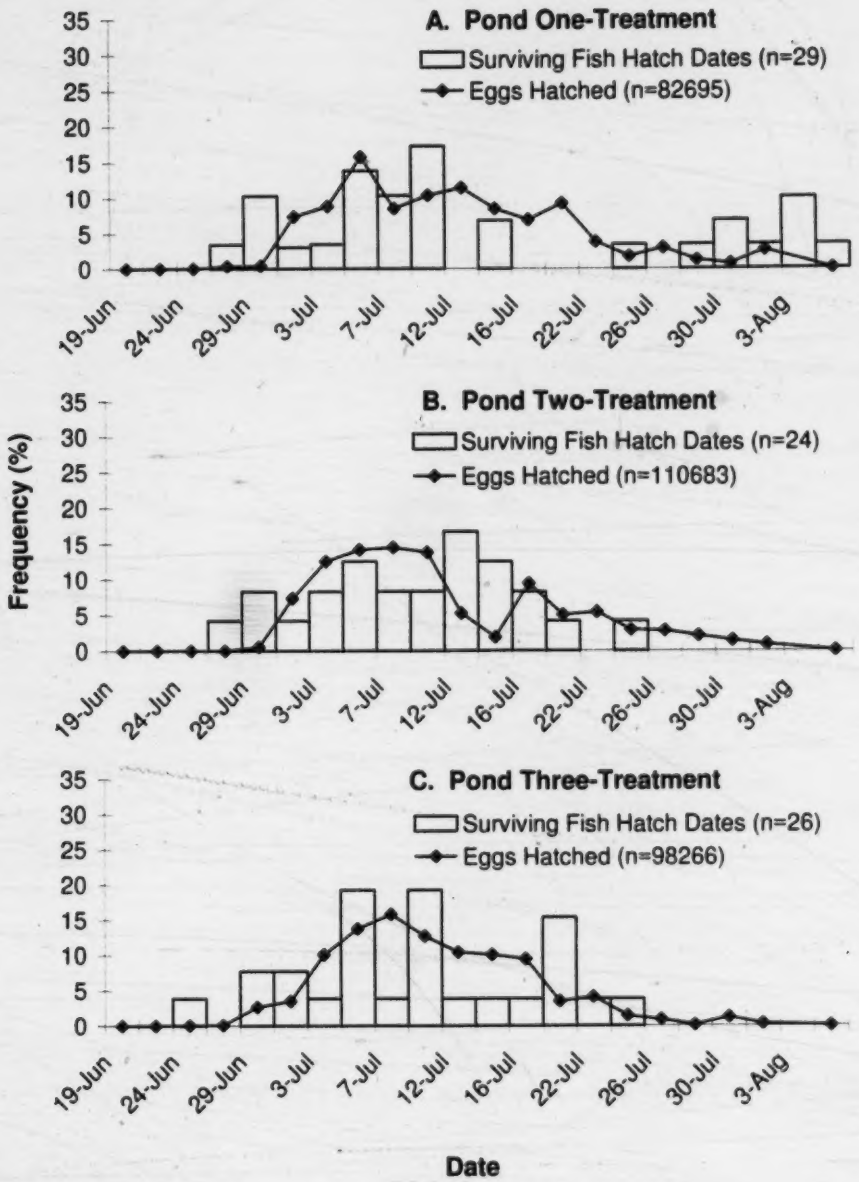

Fig. 3-16. Treatment pond sides' observed egg hatching distributions compared with hatching distributions calculated from a subsample of otoliths from surviving fish.

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4. THE INFLUENCE OF GROWTH AND BODY SIZE ON AGE-0 FATHEAD MINNOW (*PIMEPHALES PROMELAS*) MORTALITY IN NORTHERN POPULATIONS

4.1 INTRODUCTION

Variation in fish population size can largely be attributed to processes (ie., production of eggs and mortality in the egg, larval and early juvenile stages) that act in the first year of life (Sissenwine 1984). In temperate freshwater ecosystems this critical year can be divided into two distinct periods, summer and winter, characterized by differences in temperature and resource availability (Shuter et al. 1980; Shuter and Post 1990). In the summer, both predation and starvation are important sources of mortality for young fish; however, in winter, when food availability is low and feeding activity is slowed by colder water temperatures, starvation is the principal source of mortality. Although, the relative contributions of key sources of mortality for young fish differ between summer and winter, body size can influence mortality within both periods. Larger fish are less vulnerable to predation (Post and Prankevicius 1987; Rice et al. 1987; Miller et al. 1988; Crowder et al. 1992) and starvation (Frank and Leggett 1982; Miller et al. 1988). Therefore, factors that affect growth rates in the summer growing season, such as food availability and temperature, can influence overall size-dependent mortality in both the summer and winter periods.

Starvation may be particularly important as a source of mortality during the switch from endogenous to exogenous feeding, referred to as the critical period (Mills 1991). After a fish depletes its yolk supply, it must acquire sufficient food exogenously within a certain period of time, after which the effects of starvation cannot be reversed

(McGurk 1984; Miller et al. 1988; Goshorn and Epifanio 1991). Therefore, food availability is critical during this period.

Since surplus energy in young fish is allocated to growth (Fritz et al: 1990; Diana 1995), greater food availability should increase growth rates and hence body size once exogenous feeding begins. Since larger fish have increased reactive distances (visual acuity) and swimming speeds (Yates 1983; Fernald 1988) and are therefore are better able to capture food and avoid predators, they should be less susceptible to mortality due to starvation and predation. Increased growth rates should thus decrease the time spent in smaller sizes associated with higher mortality rates (Rice et al. 1987; Bailey and Houde 1989). Therefore, in the first summer of life, faster growth in young fish resulting from increased food availability should decrease mortality due to both starvation and predation.

Faster growth in the summer may also influence mortality in the winter period, since survival of young fish over winter can also be directly related to body size (Toneys and Coble 1979; Post and Evans 1989; Smith and Griffith 1994; Cargnelli and Gross 1996). In the winter, food availability is low and feeding activity in fish is slowed by cold temperatures; therefore, the key source of mortality in this period is starvation (Shuter et al. 1980; Shuter and Post 1990; Diana 1995). To survive, fish must rely on stored energy. Since smaller individuals have lower energy reserves and higher mass-specific standard metabolic rates (Brett and Groves 1979; Shuter and Post 1990), these fish will deplete their energy reserves more quickly than larger fish. Because shorter summers decrease the time available for growth and longer winters increase the time a fish must survive a period of resource scarcity, northern populations should

experience greater over-winter mortality compared with southern populations within a species' range (Shuter and Post 1990).

To assess the importance of food availability on growth and survival of age-0 fish in the summer period, I stocked enclosures with newly hatched fathead minnows (*Pimephales promelas*) and compared growth and survival between four different treatments (food: high and low; predators: present and absent). To examine the effect of body size achieved by the end of the summer on overwinter mortality, I sampled two populations of age-0 fish in the fall and again in the spring to determine if overwinter survival is dependent on body size attained during the summer growing season.

Taken together, the objective of both studies was to determine the contribution of food availability and size-dependent mechanisms to age-0 fathead minnow mortality and subsequent population size. If these mechanisms are shown to be important, then understanding fluctuations in their intensity is crucial for predicting year-class strength and the responses of fish population size to anthropogenic factors, such as global climate and land-use changes, that may affect these mechanisms.

4.2 METHODS

4.2.1. Natural History

Fathead minnows (*Pimephales promelas*) are short-lived (≤ 3 years old), small-bodied cyprinids (Markus 1934; Scott and Crossman 1973). They occur throughout central North America, with their northern distribution limited to the Alberta-Northwest Territories border (Scott and Crossman 1973). In northern Alberta, fathead minnows inhabit shallow lakes and ponds that often lack other fish species (Robinson and Tonn 1989). Therefore, conducting experiments in ponds and pond enclosures in the absence of other fishes is applicable to their ecology.

In these single-species situations, adult fathead minnows will feed on their young (eggs and juvenile stages) and, therefore, may be an important source of mortality for age-0 fish (Vandenbos 1996). Fathead minnow diets may also include plant material (algae and macrophytes), detritus, small crustaceans and insects (Scott and Crossman 1973; Held and Peterka 1974; Tallman et al. 1984; Price et al. 1991).

This species commences spawning when water temperatures reach 15.6°C – 17.8°C (generally late-May or early-June in northern Alberta) and continues until August (Scott and Crossman 1973). Since eggs are deposited on the underside of floating objects, such as logs, lily pads, and rocks (McMillan and Smith 1974), embryos can easily be collected and hatched in the laboratory (Denny 1987). Adults and juveniles are also easily collected with minnow traps and by electrofishing, respectively.

4.2.2 Summer Growth and Survival

4.2.2.1 Experimental Design

The enclosure experiment initially followed a 2 x 2 factorial design: predators present and absent and high and low food levels (but see below). Twelve enclosures were divided into three replicate blocks of four. The four enclosures in a replicate block consisted of two contiguous rows of two enclosures. The three replicates of four enclosures (replicate blocks) were adjacent to one another but separated by a distance of 1.5 m. The 12 enclosures (2.4 m (width) x 2.4 m (length) x 0.6 m (depth)) were located within an experimental pond at the Meanook Biological Station, Athabasca, Alberta (54°37'N, 113°35'W). Sides of impermeable black tarpaulin, fastened onto wooden frames, prevented water flow between enclosures. The bottom of the tarpaulin was buried into sandy sediment, exposing the enclosure bottoms to these sediments. All four treatments were randomly assigned to the four enclosures within a replicate block.

4.2.2.2 Stocked Age-0 Fish

Larval fish were obtained from eggs collected in July from a nearby unnamed natural pond (53°15'N, 113°18'W). Spawning substrata, floating wooden boards covered with black tarpaulin, were anchored in positions along the eastern shoreline of the pond where most fathead minnows spawned (personal observation). Two days later, any egg batches spawned on the boards were removed by cutting off the area of black tarpaulin containing eggs. The pieces of tarpaulin containing egg batches were then tacked onto smaller boards, submerged in coolers containing aerated pond water, and transported to the field station. At the station, the pieces of tarpaulin were placed in buckets containing aerated well water. Ten buckets, each containing 1-2 egg batches

(ca. 160 – 900 eggs per bucket), were kept outside to expose eggs to the natural photoperiod (16L:8D) and temperature (15 to 19°C). Buckets were drained daily by half and topped up with fresh water; any eggs covered with fungus were removed with tweezers. Eggs hatched within seven days and larvae were continually removed until a sufficiently large hatch occurred to ensure that larvae used in the experiment were all <1 d old.

Once a large hatch occurred, a total of 11, 400 day-0 larvae, combined from 10 egg batches, were divided equally among 12 buckets (950 / bucket). This provided an initial stocking density of 0.27 fish / L, within the range of initial age-0 fish obtained from a pond study (personal observation). Fifty larvae from each bucket were videotaped, using a COHU high performance CCD video camera, a Panasonic color video monitor (model CT-1331Y) and Leica (model MZ 6) dissecting microscope (magnification=6.3x), for later morphometric analysis. The fish were videotaped in a petri dish containing water (1 mm depth) and then returned to their respective buckets. All fish were then transferred to the enclosures. Two replicates were started on July 9, 1996 and the remaining replicate started on July 10th, 1996. To obtain standard length from the videotapes, an Optimas 5.2 image analysis system, a Panasonic color video monitor CT-1331Y and a Panasonic AG-1260 Super 4-head VHS video recorder were used. The image of each videotaped fish was frozen on the video monitor using Optimas and lengths were traced from the head to the end of the notochord. Images were calibrated every 25 fish using a videotaped ruler.

4.2.2.3 Treatments

For treatments with predators, adult fathead minnows were introduced at densities of 1 fish / m^3, similar to densities found in nature (W.M. Tonn, personal communication). However, during the 45-d study, adult mortality was substantial (up to 67%) and not equal among enclosures; as a result, data from the predator treatment could not be reliably interpreted. Since lengths (P>0.8), masses (P>0.3), and total numbers of surviving age-0 fish (P>0.8) did not differ between the two predator "treatments" (predators present and absent) within both the high and low food treatments (one-way ANOVA), predator "treatments" were pooled within each food treatment. Therefore, comparisons were made only between the two levels (high and low) of the food treatment, with six replicates for each treatment.

High food treatments received supplemental food consisting of brine shrimp, *Artemia* nauplii, which were cultured on site and harvested following guidelines outlined by Environment Canada (1992). Nauplii were rinsed with freshwater in buckets with 20 µm screen bottoms. The nauplii retained in the buckets were resuspended in freshwater. Every day ca. 300 ml of the resuspended brine shrimp was added to each high food treatment enclosure. No supplementary food was added to the low food treatments.

At the end of 45 days (August 22, 1996), surviving juveniles were collected using an electrofishing unit and dipnet and preserved in 95% ethanol. Survival was quantified by counting all fish collected from each enclosure. A subsample of approximately 50 fish per enclosure were measured (standard length) and weighed. Growth rates (G_L) (mm / day) were calculated as:

$$G_L = (L_2 - L_1)/(T_2 - T_1)$$

where L_2 is the average final (Day 45) standard length (mm), L_1 is the average initial standard length (mm), and T_1 and T_2 are the initial and final dates of the study period, respectively.

4.2.3 Overwinter Mortality Study

To determine if overwinter survival is selective with respect to size, length-distributions of age-0 fathead minnows from two experimental populations were compared before and after the winter period. Two experimental ponds (325 m^2 surface area, 1.5 m maximum depth) located at the Meanook Biological Research Station had been stocked in the spring of 1995 with adult fathead minnows. Spawning substrate was provided and fish commenced spawning in late May (Vandenbos 1996). Adult fish were removed from these ponds in September (1995) using 10 minnow traps per pond, set for 24 hour periods until no further fish were caught in the traps. Samples of age-0 fish were acquired two weeks prior to ice-up (September 16, 1995; water temperature = 6°C) from ca. eight 3 m sweeps per pond with an electrofishing unit and dip net (1 mm mesh size). All fish collected were preserved in 95% ethanol and measured (standard lengths) to within 1 mm and weighed (0.1 g). On May 2, 1996 (one week after ice-off; water temperature=5°C) each pond was seined until subsequent seine hauls produced fewer than two fish. Fish were preserved in 95% ethanol and samples from each pond were measured for length and mass.

4.2.4 Data Analysis and Statistics

Statistical analysis was conducted with SPSS Base 8.0 (SPSS Inc. 1997). Analyses are outlined in Gravetter and Wallnau (1992). Type I error probability of 0.05 was used to judge the significance of all statistical tests. For the summer growth and

survival experiment, initial lengths of stocked day-0 fish were compared among enclosures with one-way analysis-of-variance (ANOVA). After 45 days average total numbers, lengths, masses, growth, and mortality rates were compared between high and low food enclosures with ANOVA, with the three replicate blocks of four enclosures used as a fixed (blocking) factor. For the overwinter mortality study, length distributions of fish measured before and after the winter period were compared for each pond with a Kolmogorov-Smirnov two-sample test (Sokal and Rohlf 1981). Mean lengths and masses of fish before and after the winter period were compared with one-way ANOVA.

4.3 RESULTS

4.3.1 Summer Growth and Survival Experiment

Within one week of the start of the experiment, a hole formed in one enclosure for the high food treatment. Although the enclosure was repaired and some age-0 fish remained, this enclosure was excluded from all further analyses.

Initial stocking densities of age-0 fish were identical among enclosures (see 4.2.2.2 Stocked Age-0 Fish). Based on the videotaped subsample of 50 newly hatched larvae, initial lengths of stocked age-0 fish were not significantly different between each enclosure ($F_{10, 538} = 1.67$, $P = 0.08$; Table 4-1).

Average daily water temperature in the pond containing the enclosures was 19.8°C and ranged from 16.8°C to 23.4°C (A. Danylchuk unpublished data) (Fig. 4-1).

More age-0 fish survived in the high food than in the low food treatments (Table 4-1). Lengths and masses and growth of surviving age-0 fish were also greater in the high food than in the low food treatments (Table 4-1).

4.3.2 Overwinter Mortality Study

In both ponds length distributions differed between the fall (1995) and spring (1996) (Kolmogorov-Smirnov two sample test, pond one: Z=7.4, P<0.001; pond two: Z=4.8, P<0.001) (Fig. 4-2). Lengths before ice-up averaged 19.9 ± 0.49 mm (mean ± SE) and 24.4 ± 0.56 mm for pond one and two, respectively, compared with average lengths after ice-out of 32.2 ± 0.29 mm and 31.4 ± 0.47 mm. Masses before ice-up averaged 77.14 ± 8.44 mg and 136.9 ± 8.95 mg for pond one and two, respectively, compared with average masses after ice-out of 297.5 ± 9.1 mg and 278.8 ± 14.6 mg. Length, mass, and condition (Fig. 4-3) were greater in fish surviving to spring compared

with fish measured in the fall for both pond one (length: $F_{1,330}$=530.3, P<0.001; mass: $F_{1,329}$=257.0, P<0.001; condition: $F_{1,329}$=9.8, P=0.002) and pond two (length: $F_{1,184}$=76.35, P<0.001; mass: $F_{1,183}$=77.3, P<0.001; condition: $F_{1,183}$=11.2, P=0.001).

4.4 DISCUSSION

4.4.1 Summer Growth and Survival Experiment

In the enclosure experiment, food availability significantly influenced mortality of age-0 fathead minnows; mortality was approximately 2-fold greater in the low-food than in the high-food treatment. Hjort (1914) first proposed the hypothesis that variability in mortality in young fish is due primarily to starvation (poor nutrition). Since that time, several studies have found evidence for starvation mortality both in the laboratory (Goshorn and Epifanio 1991) and in the field (Frank and Leggett 1986; Goshorn and Epifanio 1991; Michaletz 1997).

Mortality due to starvation in young fish has been thought to be most significant during the switch from endogenous to exogenous feeding, known as the critical period (Hjort 1914). If sufficient food is not available at this time, a fish will reach a point-of-no-return (PNR). Even if food availability increases after the PNR is reached, a fish will be too weak to feed and therefore will die of starvation (McGurk 1984; Goshorn and Epifanio 1991).

Several studies, however, have not found conclusive evidence for the existence of the PNR (Rice et al. 1987; Miller et al. 1988). Miller et al. (1988), in compiling data on numerous species, suggested that newly hatched fish are more susceptible to death by starvation simply because this was when they were smallest. Therefore, they concluded that body size might be the most important factor influencing susceptibility of fish to starvation. Smaller fish may be more vulnerable to starvation because they have smaller energy stores and higher mass-specific basal metabolism (Brett and Groves 1979; Shuter and Post 1990). In addition, decreased visual acuity (Fernald

1988) and swimming performance (Yates 1983) associated with smaller body sizes may also decrease the ability to capture prey. Thus, the amount of time a fish spends in a small size class should greatly influence its chances for survival in early life. It follows then that growth rates experienced by newly hatched fish should influence their susceptibility to starvation since faster growth rates will decrease the length of time fish spend in smaller, more vulnerable sizes.

In my experiment, food availability significantly influenced growth rates of age-0 fathead minnows. Average growth rates were 0.18 mm/day and 0.42 mm/day for the low and high food enclosures, respectively. These are comparable to rates (0.27 – 0.33 mm/day) calculated from daily growth increments on otoliths in a study I conducted in experimental ponds in the same season (Chapter Two). Smith et al. (1978) also found that length and mass of fathead minnows increased with increased food availability, although fish in their study were more than 21 days old. The difference in growth rates between the high and low food enclosures, by influencing the time a fish spends in a smaller size class, could have therefore further influenced overall mortality due to starvation.

Growth rates in young fish can also influence mortality due to predation, since slow growth rates extend the time young fish are susceptible to predators (Rice et al. 1987; Bailey and Houde 1989). In addition, the poorer visual acuity and reduced swimming abilities of smaller fish (Yates 1983; Fernald 1988) make them more vulnerable to predation. Although my study was initially designed to observe the effects of predation and its interaction with nutrition, this source of mortality could not be assessed due to the differential mortality of the adults (predators). However, the

significantly greater growth rates of age-0 fish in the high versus low food treatments suggest that mortality due to predation might be lower when food is more abundant.

Although adult fathead minnows can feed on their own young (larval and juvenile stage), they are gape-limited cannibals; they cannot eat fish larger than 11 – 19 mm, depending on the size of the adult (Vandenbos 1996). In my study, at the end of 45 days, the average age-0 fish (standard length: 12.9 mm) in the low food treatments would still be vulnerable to most size-classes of adults. In contrast, the average fish (standard length: 23.8 mm) in the high food treatments exceeded the maximum size (19 mm) that even the largest fathead minnow tested (>70 mm) could ingest (Vandenbos 1996). It is therefore reasonable to suggest that when food is more abundant, age-0 fathead minnows should experience less mortality due to predation by adult fathead minnows as a result of faster growth.

4.4.2 Overwinter Mortality Study

Age-0 overwinter mortality was strongly selective for smaller body sizes; mean lengths were 7-12 mm greater in the spring compared to the fall sampling periods. In both study ponds, fish smaller than 21 mm did not survive the winter period. The differences observed in both average length and length-frequency distribution between the two sampling periods can be primarily attributed to size-selective overwinter mortality rather than overwinter growth. In cyprinid fish, growth generally ceases below temperature thresholds that range from 12 – 15°C (Mann 1991), although thresholds for species found in more northern latitudes have been reported as low as 7°C (Mann 1991).

In field studies, increases in mean lengths or changes in the length-frequency distributions have been provided as evidence for size-selective mortality. Such studies have observed size-selective overwinter mortality for bluegill (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*) (Toneys and Coble 1979), rainbow trout (*Oncorhynchus mykiss*) (Smith and Griffith 1994) and yellow perch (*Perca flavescens*) (Post and Evans 1989) populations. Laboratory studies have also provided evidence of size-selective mortality in age-0 fish under simulated winter conditions for smallmouth bass (*Micropterus dolomieui*) (Oliver et al. 1979; Shuter et al. 1980) and yellow perch (*Perca flavescens*) (Post and Evans 1989).

Smaller fish may experience higher overwinter mortality due to their relatively high mass-specific standard metabolism and their relatively low energy reserves (Brett and Groves 1979; Shuter and Post 1990). In the winter period, since food availability is low and fish generally do not feed at colder temperatures (Shuter et al. 1980; Shuter and Post 1990; Diana 1995), smaller fish should deplete their energy reserves faster than larger fish (Post and Evans 1989). A consequence of this observation is that changes in factors that influence growth of age-0 fish during the summer period and therefore influence body size prior to freeze-up will strongly influence the intensity of size-selective starvation during the winter period.

Two key factors that influence growth and body size of age-0 fish during the summer period and hence body size prior to the winter period are food availability (see above) and temperature. Water temperature plays an important role in the physiological and physical reactions within a fish, since fish are ectothermic poikliotherms (Diana 1995). Several studies have shown that warmer temperatures increase growth rates in

age-0 fish (Shuter et al. 1980; Mooij et al. 1994; Michaletz 1997), up to a temperature optimum, with growth decreasing beyond the optimum (Diana 1995).

However, the size distribution at the end of the growing season is not the only factor that determines the degree of overwinter mortality. Both the length of the winter season and winter temperatures are also important factors. Longer winters increase the time a fish must rely on its energy reserves to maintain its basal energy requirements and therefore increase the chance that a fish will deplete these energy reserves (Oliver et al. 1979; Post and Evans 1989). Temperature in the winter period can also influence mortality of age-0 fish. Smith and Griffith (1994) found that mortality of age-0 rainbow trout over the winter period was greater at colder water temperatures.

In contrast, food availability in the winter period may not be an important factor for overwinter survival (Shuter and Post 1990). Even if food is abundant, slowed feeding activity may still prevent fish from acquiring sufficient food to maintain basal energy requirements. In a laboratory study, fish lost condition even though food was supplied *ad libitum* under simulated winter conditions (Post and Evans 1989).

4.4.3 Population Variability and Species Range

Near the northern limit of a species' range the summer period shortens, winters lengthen, and temperatures are colder. As a result, fish in these areas attain smaller lengths during their first growing season and have to rely on stored energy reserves for longer periods of time over the winter. More northerly populations, therefore, should experience greater size-selective overwinter mortality. Beyond the distributional limit, the combination of short summers and long winters prevent age-0 fish from attaining sufficient body size in the summer to survive the winter period. At this point

recruitment ceases and a population is no longer viable. Climate is therefore the principal factor that ultimately determines the degree of size-selective overwinter mortality and hence the northern limit in a fish species' distribution. In the genus *Pimephales*, the importance of climate is evident in their northern distribution, which follows mean annual temperature isotherms (Shuter and Post 1990).

Previous studies on size-selective overwinter mortality have been conducted on species at more central and southern locations within their ranges, with moderate overwinter time periods (reviewed in Shuter and Post 1990). Toneys and Coble (1979) found no evidence for size-selective mortality in several fish species, including the fathead minnow. Laboratory studies conducted under simulated winter conditions characteristic of more southerly latitudes similarly found no evidence for size-selective mortality in several species, including green sunfish, largemouth bass, brook trout and yellow perch (Toneys and Coble 1979).

In northern Alberta, fathead minnows occur near the northern edge of their distribution (Scott and Crossman 1973). These populations experience shorter and cooler summer growing seasons and longer overwinter periods than more southern populations and, as a result, size-selective overwinter mortality should be more intense. The results from my study are the strongest empirical demonstration of size-selective overwinter mortality in more northern populations and therefore support predictions based on theory.

4.4.4 Population Variability in Response to Climate and Land-Use Change

Since climate is the main factor that determines the degree of size-selective overwinter mortality and the limit to a species northern distribution, it follows that

changes in climate can alter both of these. In response to increased anthropogenic activities, chiefly the burning of fossil fuels, a doubling of atmospheric concentrations of CO_2 and a resultant increase in global temperatures have been predicted (Hansen et al. 1984). A recent study of global temperatures over the period 1965-1995 revealed that the greatest seasonal warming had occurred in winters at northern latitudes (Hansen et al. 1996). If such warming affected the length of winters, this climate change should increase year-class strength for fathead minnows in more northerly populations, since the intensity of size-selective overwinter mortality should decrease. Over time, this would shift the northern distributional limit further north (Shuter and Post 1990).

In northern Alberta, increased logging and agriculture can further influence mortality of age-0 fathead minnows. The predicted impacts of both activities is an increase in nutrient loads and subsequent productivity in the adjacent aquatic ecosystems (Sharpley and Smith 1993; Schlesinger 1997; Carpenter et al. 1998). Based on results obtained in my study, an increase in the food availability for age-0 fathead minnows should increase growth and decrease mortality in the summer period. The larger body sizes attained prior to winter should reduce overwinter mortality for this species. Provided the increase in productivity does not substantially deplete dissolved oxygen levels under the ice over the winter period, increased nutrient loading in aquatic systems within northern Alberta should increase year-class strength of fathead minnows.

4.4.5 Conclusions

I have argued that the prediction of year-class sizeof fathead minnow populations in northern Alberta should consider mechanisms acting both in the summer

and winter. In the summer, food availability can influence growth rates and mortality due to starvation. Faster growth rates may further reduce mortality due to predation. Since fathead minnow adults have been shown to cannibalize their own young (Vandenbos 1996), predation should also be an important source of mortality even in ponds and lakes containing only fathead minnows, a common occurrence in ponds and lakes in northern Alberta (Robinson and Tonn 1989).

As revealed in my study, age-0 fish in populations at higher latitudes may experience substantial size-selective mortality over winter due to a) shorter growing seasons and cooler summer temperatures that result in smaller age-0 fish prior to the winter season, and b) longer winter seasons, which cause proportionately more age-0 fish to deplete their energy stores and die from starvation.

Northern fish populations, such as the fathead minnows from boreal Alberta that were the subject of my study, are expected to experience considerably altered environments as a result of global and regional changes in climate and land-use brought about by human activities. The consideration of mortality mechanisms acting both in the summer and winter should allow us to better predict the response of year-class strength in fish populations to such anthropogenic changes.

Table 4-1. Average (± SE) total numbers per enclosure (N), standard lengths (SL), and masses of age-0 fathead minnows at the start of the experiment in July (all fish = 0 days old) and at the end of the experiment in August (all fish 45 days old) and growth for fish throughout the 45-d study period, for high and low food treatments. More age-0 fish survived to the end of the experiment (ANOVA, $F_{1,2}$=9.03, P=0.04) in high versus low food enclosures. Surviving fish in high food enclosures also had greater lengths ($F_{1,2}$=1344, P<0.001), masses ($F_{1,2}$=281.7, P<0.001), and growth rates ($F_{1,2}$=287.0, P<0.001) than fish in low food enclosures.

	Initial (July)		Final (August)			Growth
Treatment	N	SL (mm)	N	SL (mm)	Mass (g)	(mm/day)
High Food [a]	950	4.88 (0.014)	307.8 (34.9)	23.8 (0.38)	0.15 (0.01)	0.42 (0.01)
Low Food [b]	950	4.89 (0.011)	182.2 (25.5)	12.9 (0.68)	0.02 (0.004)	0.18 (0.02)

[a] n=5 for each initial SL and final SL, mass, and growth
[b] n=6 for each initial SL and final SL, mass and growth

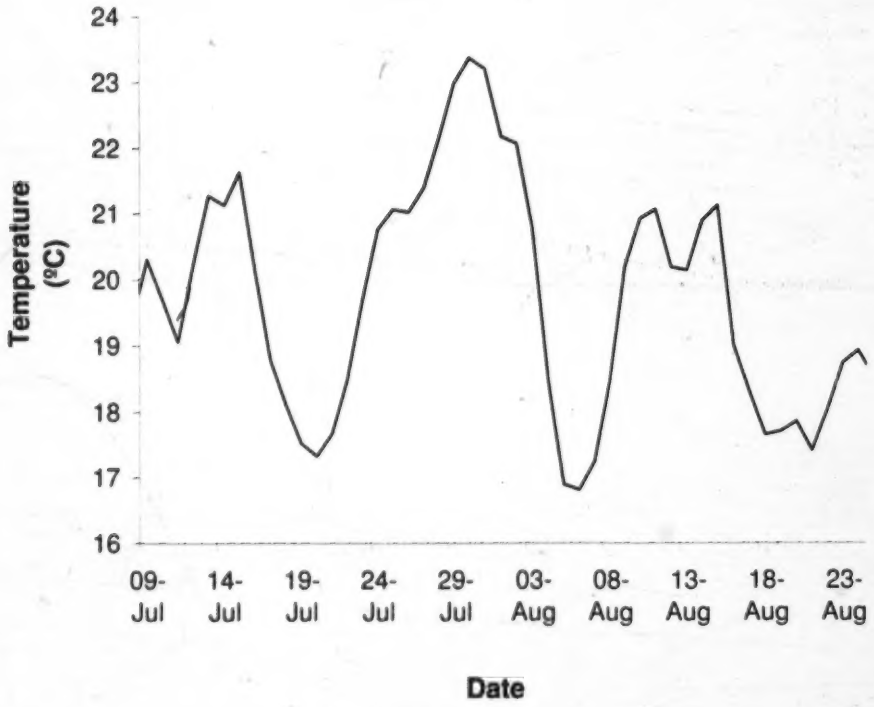

Fig. 4-1. Average daily water temperatures at 0.3 m in pond containing enclosures, from July 9 – August 23, 1996. Data obtained from A. Danylchuk (unpublished).

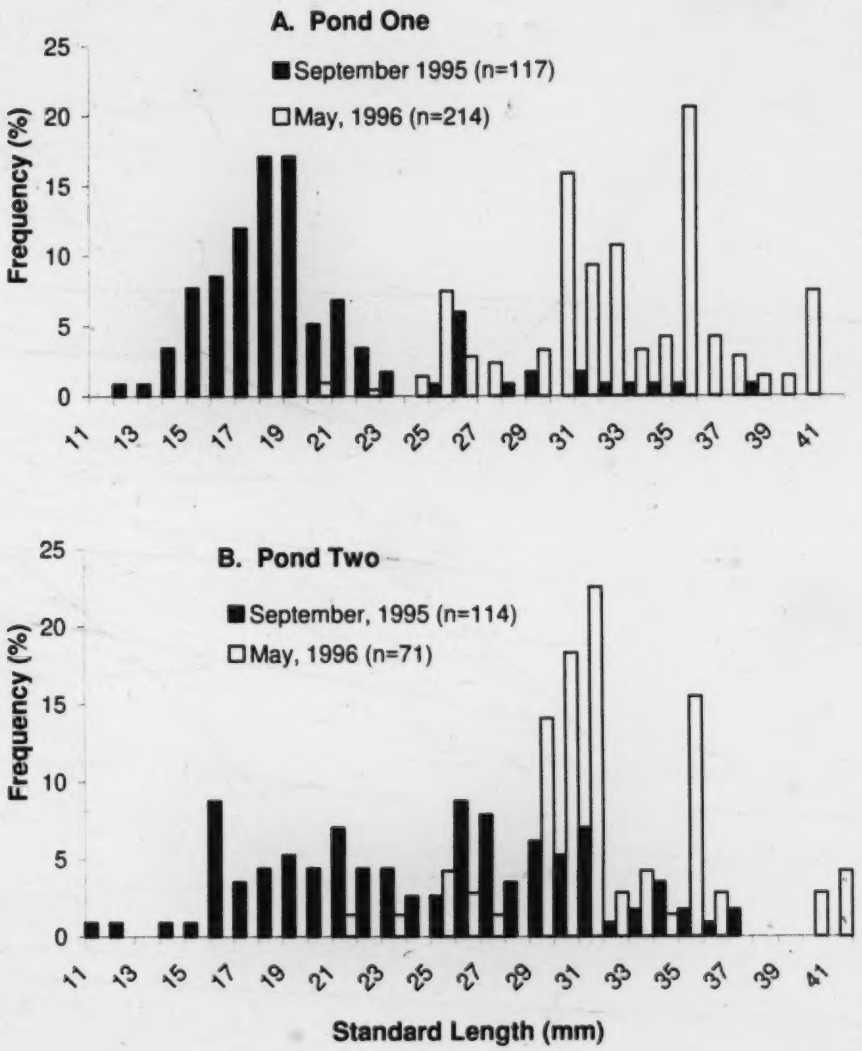

Fig. 4-2. Length-frequency distributions for age-0 fish just before ice-up (September 1995) and the following spring (May 1996) for A. pond one and B. pond two, in the overwinter mortality study.

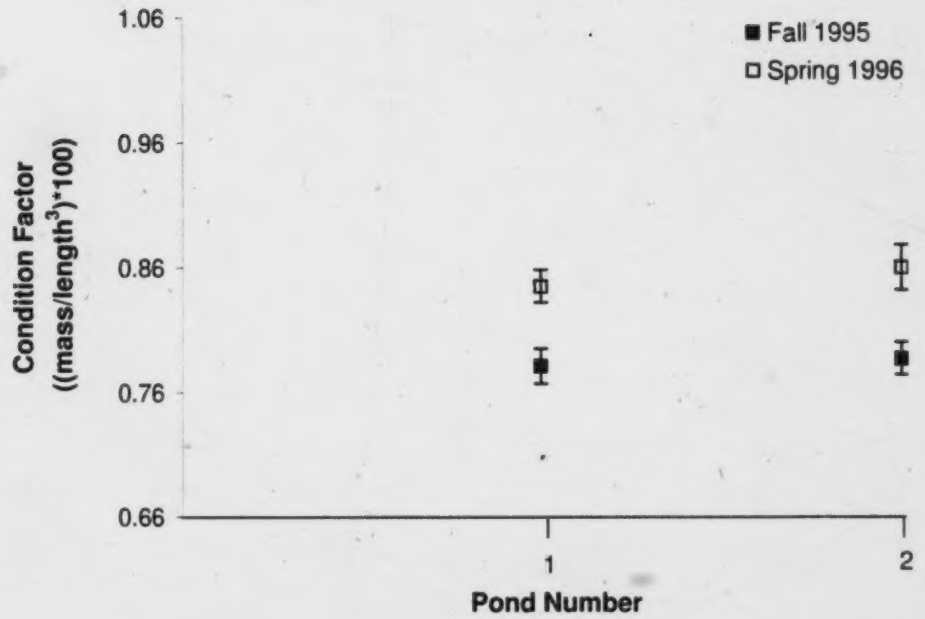

Fig. 4-3. Condition index for age-0 fish in pond one and pond two prior to ice up (September 1995) and after spring thaw (May 1996), for the overwinter mortality study. Bars represent ± SE.

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5. GENERAL DISCUSSION

5.1 KEY RESULTS

Chapter Two: In my pond study, nutrient enrichment strongly increased recruitment of age-0 fathead minnows (*Pimephales promelas*). Increased egg production and decreased age-0 fish mortality both contributed to the number of age-0 fish surviving to the end of their first summer of life. Lengths of age-0 fish were greater in treatment versus reference pond sides; with their length-frequency distributions skewed to larger sizes in the treatment pond sides. **Chapter Three:** Otoliths of age-0 fathead minnows provided a useful tool to determine more clearly the mechanisms influencing survival of the age-0 fish. My study determined specifically for the fathead minnow that age-0 fish lay down otolith increments on a daily basis. This result represents the first assessment of daily increment formation for this species. Using this information and hatch-date distributions of surviving fish, calculated from otoliths, I determined that fish subject to an overall increase in food availability (nutrient enhanced treatments) exhibited no selective mortality with respect to hatch date. In contrast, fish that did not experience an overall increase in food availability (no nutrients added) exhibited selective mortality with respect to hatch-date; the surviving fish were drawn disproportionately from the second half of the spawning season when natural food availability was the greatest. **Chapter Four:** In the enclosure experiment, food availability significantly increased growth rates and survival of age-0 fish, and in a separate pond study, body size prior to the winter period strongly influenced overwinter mortality of age-0 fish.

These results will be discussed below as they relate to a) mechanisms influencing fish population dynamics and b) the predicted effects of increased global and regional anthropogenic disturbances on fish populations.

5.2. DISCUSSION

Through its effects on food availability for fathead minnows, increased nutrient loads significantly enhanced recruitment of age-0 fish to the end of the first growing season (summer period). Small-scale aquaculture studies (Fox et al. 1989; Culver et al.1993; Myers et al. 1996; Tice et al. 1996) and large-scale ecosystem studies (Colby et al. 1972; Nakashima and Leggett 1975; Mills 1985; Mills and Chalanchuk 1987) have similarly reported enhanced recruitment of young fish in response to the addition of nutrients to ponds and lakes. In these studies, decreased mortality in the larval and juvenile stages was suggested as the principal mechanism influencing recruitment. However, in addition to decreased mortality in the larval and juvenile stages, my study was the first to show that a greater number of eggs laid by adult fathead minnows in nutrient enriched pond sides also contributed to enhanced recruitment of age-0 fish. In previous studies, recruitment mechanisms in the egg stage (number of eggs laid, egg mortality, number of eggs hatched) have not been assessed, since, in most systems, observation and accurate quantification of eggs from laying to hatch is difficult if not impossible.

One approach that has yielded greater understanding of the specific mechanisms controlling survival in age-0 fish is the shift of the study unit from the population to the individual (Post and Prankevicius 1987; Rice et al. 1987; Crowder et al. 1992; Cargnelli and Gross 1996; Gleason and Bengtson 1996). Specifically, since the average fish dies within the first weeks of life, comparing the characteristics of survivors with the average characteristics of the cohort sampled at earlier dates can help identify the mechanisms that influenced survival. The discovery of daily growth increments on

otoliths of young fish (Panella 1971, 1974) has been critical in the development of this approach. I provided the first evidence of daily increment formation for the otoliths of the fathead minnow. This step is essential for the use of otolith techniques in the back-calculation of fish lengths (Campana 1990) and hatch dates (Campana and Neilson 1985; Rice et al. 1987; Campana and Jones 1992) in the species being studied.

In my pond study, the successful use of otoliths in the investigation of mechanisms influencing age-0 fish survival suggests the practicality of using this method in studies on natural fathead minnow populations. If I had not used otolith analysis, the importance of intra-seasonal variability of food availability, in addition to increased total food availability, to the survival of age-0 fish would not have been recognized. The influence of intra-seasonal variation in factors such as food availability, temperature, and predator densities on the intensity of mortality within a growing season has also been reported for other species (Crecco and Savoy 1985; Rice et al. 1987; McGovern and Olney 1996; Bulak et al. 1997). Similar to my study, when mortality is particularly intense at certain times within a season, survival of fish has been demonstrated to be selective with respect to hatch-date (Crecco and Savoy 1985; Rice et al. 1987; McGovern and Olney 1996; Bulak et al. 1997).

In addition to mechanisms acting on age-0 fish in the first growing season (summer period), size-dependent overwinter mortality can also contribute to recruitment in fish (Shuter and Post 1990, Post and Evans 1989). Size-dependent overwinter mortality should be particularly important for populations in northern latitudes because the period of resource abundance and suitable temperatures for feeding and growth is shorter compared to more southern latitudes (Shuter and Post 1990). As a result, in

northern populations fewer fish should be able to grow to the minimum body size necessary to endure overwinter starvation, thus increasing the intensity of size-selective overwinter mortality in these regions.

My overwinter mortality study is the strongest empirical demonstration that overwinter mortality in northern fathead minnow populations is size-selective and therefore supports the predictions based on theory. These results complement a study conducted by Toneys and Coble (1979) that found no evidence for size-selective overwinter mortality for more southern fathead minnow populations. The results from both my study and Toneys and Coble's (1979) study suggest that variation in overwinter mortality in fathead minnows does occur. However, future studies need to be conducted to determine, specifically the role of latitude on the intensity of overwinter mortality.

Since overwinter mortality in northern fathead minnow populations is strongly selective for smaller individuals, it follows then that factors that influence age-0 fish length prior to the winter period should influence the intensity of overwinter mortality and, hence, population size. In my study on fathead minnows, an increase in nutrient loading was shown to be one factor that can increase the body size of age-0 fish to the end of their first summer of life. In a separate enclosure experiment, direct increases in food availability for age-0 fatheads also resulted in increased body size after 45 days. Therefore, increases in nutrient loads to aquatic ecosystems, predicted to occur due to global climate (Waggoner 1990; Carpenter et al. 1992) and land-use changes (Schlesinger 1997), should result in increased body sizes for age-0 fathead minnows prior to the winter period. More fish should survive over the winter period and, as a

result, recruitment and population size should increase. A key assumption in this argument is that the increased productivity in the summer period does not increase winter oxygen depletion and result in increased winter-kill (Schindler 1990). The effect of enhanced nutrient loading on dissolved oxygen levels becomes the logical next step for future study, and is discussed further below (see Section 5.3).

Beyond a species distributional limit, shorter summer growing seasons and longer winters prevent fish from attaining sufficient body sizes in the summer to survive the winter period. At this point a population is no longer viable (Shuter and Post 1990). However, if factors that influence body size of young fish prior to the winter period change, such as temperature and food availability (as previously described), then it follows that the northern distributional limit for a species might also change.

Shuter and Post (1990) modeled the shift in northern distributional limits for smallmouth bass (*Micropterus dolomiei*) and yellow perch (*Perca flavescens*) populations based on predictions of global climate change. However, temperature was the only parameter in their model adjusted to reflect the predicted global climate change (Hansen et al. 1984), whereas there may also be changes in nutrient loads to aquatic ecosystems (Carpenter et al. 1992), and hence food availability (Colby et al. 1972; Milstein et al. 1995; Myers et al. 1996; Tice et al. 1996), that may affect the northern distributional limit of a fish species. In my study on fatheads, since increased nutrient loads enhanced sizes of age-0 fish prior to the winter, it follows that an additional increase in nutrient loads to aquatic ecosystems should cause a further shift of species' distributions to northern latitudes, beyond that predicted in Shuter and Post's model (1990), which considered only temperature changes.

Regional increases in nutrient loading may also result from land-clearing activities (Schlesinger 1997), such as logging and agriculture. In addition, fertilizer-use in agriculture may also contribute to increased nutrient loads to adjacent aquatic ecosystems (Sharpley and Smith 1993; Carpenter et al. 1998). An increase in these land-use practices at northern latitudes (e.g., logging of the boreal forest in northern Alberta) could contribute to the extension of a species northern distributional limit via the same nutrient loading/recruitment mechanisms described above.

5.3 FUTURE STUDIES

In natural ecosystems many biotic and abiotic factors interact to influence survival of young fish and hence population dynamics. Numerous mechanisms have been proposed to explain how these factors act (Post and Prankevicius 1987; Miller et al. 1988; Crowder et al. 1992). However, due to the complexity of natural systems, smaller-scale enclosure and pond studies are more appropriate for the initial identification of the mechanisms regulating recruitment. The ultimate goal of these types of studies is to understand and predict natural fish population dynamics by quantifying the factors and mechanisms underlying recruitment variation in fishes. In my studies, conducted in experimental enclosures and ponds, I identified and quantified mechanisms linking specific environmental conditions (increased nutrient loads and food availability) to survival of young fish.

To determine more accurately how food availability influences recruitment of age-0 fathead minnows, smaller-scale enclosure studies should be conducted. In the egg stage, the influence of increased ration size on the number of eggs laid, the time interval between subsequent spawning for an individual fish and the number of times an individual fish can spawn within a season should be evaluated. To date, studies relating to the influence of ration size on the numbers of eggs produced are poorly represented in the literature (Wootton 1977).

In age-0 fish, the influence of food availability on growth and subsequent mortality due to predation and starvation should also be evaluated (as described in Chapter Four) to quantify the relative contribution and interactions of predation and starvation to mortality of age-0 fish. Another enclosure study should be conducted to

determine if cannibalism of both eggs and young by adult fathead minnows is reduced when the availability of alternative food increases.

Although my pond study provided much insight on the mechanisms influencing survival of age-0 fathead minnows, future pond studies should be conducted to expand results obtained from my study. Replication in future pond studies should be increased to improve statistical power. The response of pond fish populations to different nutrient combinations and loads, chosen to reflect the range of nutrient changes anticipated in response to global climate and land-use changes, should be quantified. Longer-term studies, spanning several years, should also be conducted to determine if and when a new population size equilibrium is established. Further, one necessary component to understanding the interactions between population dynamics and nutrient addition has not yet been fully addressed, namely, how the intensity of overwinter mortality differs between nutrient enriched and unenriched sites. Oxygen depletion in nutrient enriched and unenriched aquatic environments also needs to be compared and its impact on fish population size quantified.

Otolith microstructural analysis is the crucial next step in the application of these pond and enclosure study results to natural populations. In the fathead minnow, this technique needs to be refined using a combination of controlled enclosure and laboratory studies. Increment formation needs to be studied for a broader range of young fish ages (<1 year old) under different temperature and food regimes. Also, the age of first increment formation needs to be verified to determine fish age with more precision. Because of the tight and, at times, weak banding observed on fathead otoliths, electron microscopy techniques should also be used to determine if increment

formation falls within or below the resolution limits of light microscopy. The combination of improved otolith microstructural techniques and the mechanistic studies described above will be necessary for understanding the response of natural fish population sizes to increasing climate and land-use change. Clearly, the successful application of this knowledge is contingent upon adequate baseline data from wild fish populations—especially those found at higher latitudes. The value of research programs whose central aim is the collection of such baseline data from natural fish populations cannot be overstressed.

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Appendix 2-1. List of zooplankton observed in experimental ponds throughout the 1996 season (May – September, 1996).

Name	MEASURED LENGTHS (µm)
Copepoda	
Calanoida	
family Diaptomidae	1200-1800
copepodite stages	800-1100
nauplii	100
Cyclopoida	
Diacyclops bicuspidatus thomasi	1000
Acanthocyclops vernalis	1000
copepodite stages	
nauplii	300
Cladocera	
Family Chydoridae	
Chydorus	200
Pleuroxus procurvatus	200
Alonella	200
Family Daphnidae	
Daphnia pulex	1000
Daphnia rosea	800
Scapholebris aurita	500
Scapholebris kingi	500
Scapholebris mucronata	400
Simocephalus	
Rotifera	
Order Ploima	
Family Trichocercidae	150
Family Branchionidae	150